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(54) Title: RECOMBINANT ANTI-VLA4 ANTIBODY MOLECULES

(57) Abstract

The present invention discloses recombinant anti-VLA4 antibody molecules, including humanized recombinant anti-VLA4 antibody molecules. These antibodies are useful in the treatment of specific and non-specific inflammation, including asthma and inflammatory bowel disease. In addition, the humanized recombinant anti-VLA4 antibodies disclosed can be useful in methods of diagnosing and localizing sites of inflammation.

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RECOMBINANT ANTI-VLA4 ANTIBODY MOLECULES

FIELD OF THE INVENTION

The present invention relates to recombinant anti-VLA4 antibody molecules, including humanized recombinant anti-VLA4 antibody molecules.

BACKGROUND OF THE INVENTION

A. Immunoglobulins and Monoclonal Antibodies

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise generally a Y-shaped molecule having an antigen-binding site towards the free end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Specifically, immunoglobulin molecules are comprised of two heavy (H) and two light (L) polypeptide chains, held together by disulfide bonds. Each chain of an immunoglobulin chain is divided into regions or domains, each being approximately 110 amino acids. The light chain has two such domains while the heavy chain has four domains. The amino acid sequence of the amino-terminal domain of each polypeptide chain is highly variable (V region), while the sequences of the remaining domains are conserved or constant (C regions). A light chain is therefore composed of one variable (V_L) and one constant domain (C_L) while a heavy chain contains one variable (V_H) and three constant domains (CH_1, CH_2 and CH_3). An arm of the Y-shaped molecule consists of a light chain (V + C_L) and the variable domain (V_H) and one constant domain (CH_1) of a heavy chain. The tail of the Y is composed of

the remaining heavy chain constant domains (CH_2 + CH_3). The C-terminal ends of the heavy chains associate to form the Fc portion. Within each variable region are three hypervariable regions. These hypervariable regions are also described as the complementarity determining regions (CDRs) because of their importance in binding of antigen. The four more conserved regions of the variable domains are described as the framework regions (FRs). Each domain of an immunoglobulin consists of two beta-sheets held together by a disulfide bridge, with their hydrophobic faces packed together. The individual beta strands are linked together by loops. The overall appearance can be described as a beta barrel having loops at the ends. The CDRs form the loops at one end of the beta barrel of the variable region.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realization of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies (MAbs) of defined specificity, Kohler et al., 1975 [1]. However, most MAbs are produced by fusions of rodent (i.e., mouse, rat) spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins.

By 1990, over 100 murine monoclonal antibodies were in clinical trials, particularly in the U.S. and especially for application in the treatment of cancer. However, by this time it was recognized that rejection of murine monoclonal antibodies by the undesirable immune response in humans termed the HAMA (Human Anti-Mouse Antibody) response was a severe limitation, especially for the treatment of chronic disease. Therefore, the use of rodent MAbs as therapeutic agents in humans is

inherently limited by the fact that the human subject will mount an immunological response to the MAb and either remove the MAb entirely or at least reduce its effectiveness. In practice MAbs of rodent origin may not be used in a patient for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. In fact, a HAMA response has been observed in the majority of patients following a single injection of mouse antibody, Schroff et al., 1985 [2]. A solution to the problem of HAMA is to administer immunologically compatible human monoclonal antibodies. However, the technology for development of human monoclonal antibodies has lagged well behind that of murine antibodies (Borrebaeck et al., 1990 [3] such that very few human antibodies have proved useful for clinical study.

Proposals have therefore been made for making non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanization" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule. The use of recombinant DNA technology to clone antibody genes has provided an alternative whereby a murine monoclonal antibody can be converted to a predominantly human-form (i.e., humanized) with the same antigen binding properties (Riechmann et al., 1988 [4]). Generally, the goal of the humanizing technology is to develop humanized antibodies with very little or virtually no murine component apart from the CDRs (see, e.g., Tempest et al., 1991 [5]) so as to reduce or eliminate their immunogenicity in humans.

Early methods for humanizing MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from

another antibody. Methods for carrying out such chimerization procedures have been described, for example, in EP 120694 [6], EP 125023 [7], and WO 86/01533 [8]. Generally disclosed are processes for preparing antibody molecules having the variable domains from a non-human MAb such as a mouse MAb and the constant domains from a human immunoglobulin. Such chimeric antibodies are not truly humanized because they still contain a significant proportion of non-human amino acid sequence, i.e., the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period, Begent et al., 1990 [9]. In addition, it is believed that these methods in some cases (e.g., EP 120694 [6]; EP 125023 [7] and U.S. Patent No. 4,816,567 [10] did not lead to the expression of any significant quantities of Ig polypeptide chains, nor the production of Ig activity without *in vitro* solubilization and chain reconstitution, nor to the secretion and assembly of the chains into the desired chimeric recombinant antibodies. These same problems may be noted for the initial production of non-chimeric recombinant antibodies (e.g., U.S. Patent No. 4,816,397 [11].

B. Humanized Recombinant Antibodies and CDR-Grafting Technology

Following the early methods for the preparation of chimeric antibodies, a new approach was described in EP 0239400 [12] whereby antibodies are altered by substitution of their complementarity determining regions (CDRs) for one species with those from another. This process may be used, for example, to substitute the CDRs from human heavy and light chain Ig variable region domains with alternative CDRs from murine variable region domains. These altered Ig variable regions may subsequently be combined with human Ig constant regions to created antibodies which are totally human in composition except for the substituted murine CDRs. Such

murine CDR-substituted antibodies would be predicted to be less likely to elicit a considerably reduced immune response in humans compared to chimeric antibodies because they contain considerably less murine components.

The process for humanizing monoclonal antibodies via CDR grafting has been termed "reshaping". (Riechmann et al., 1988 [4]; Verhoeyen et al., 1988 [13]. Typically, complementarity determining regions (CDRs) of a murine antibody are transplanted onto the corresponding regions in a human antibody, since it is the CDRs (three in antibody heavy chains, three in light chains) that are the regions of the mouse antibody which bind to a specific antigen. Transplantation of CDRs is achieved by genetic engineering whereby CDR DNA sequences are determined by cloning of murine heavy and light chain variable (V) region gene segments, and are then transferred to corresponding human V regions by site-directed mutagenesis. In the final stage of the process, human constant region gene segments of the desired isotype (usually gamma 1 for C_H and kappa for C_L) are added and the humanized heavy and light chain genes are coexpressed in mammalian cells to produce soluble humanized antibody.

The transfer of these CDRs to a human antibody confers on this antibody the antigen binding properties of the original murine antibody. The six CDRs in the murine antibody are mounted structurally on a V region "framework" region. The reason that CDR-grafting is successful is that framework regions between mouse and human antibodies may have very similar 3-D structures with similar points of attachment for CDRs, such that CDRs can be interchanged. Nonetheless, certain amino acids within framework regions are thought to interact with CDRs and to influence overall antigen binding affinity. The direct transfer of CDRs from a murine antibody to produce a recombinant humanized antibody

without any modifications of the human V region frameworks often results in a partial or complete loss of binding affinity.

In Riechmann et al., 1988 [4] and WO 89/07454 [14], it was found that transfer of the CDR regions alone (as defined by Kabat et al., 1991 [15] and Wu et al., 1970 [16] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al. 1988 [4] found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having satisfactory antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanized antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, for example, in the loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognize more complex antigens. Even so, the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

More recently, Queen et al., 1989 [17] and WO 90/07861 [18] have described the preparation of a humanized antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. They have demonstrated one solution to the problem of the loss of binding affinity that often results from direct CDR transfer without any modifications of the human V region framework residues; their solution involves two key steps. First, the human

V framework regions are chosen by computer analysis for optimal protein sequence homology to the V region framework of the original murine antibody, in this case, the anti-Tac MAb. In the second step, the tertiary structure of the murine V region is modelled by computer in order to visualize framework amino acid residues which are likely to interact with the murine CDRs and these murine amino acid residues are then superimposed on the homologous human framework. Their approach of employing homologous human frameworks with putative murine contact residues resulted in humanized antibodies with similar binding affinities to the original murine antibody with respect to antibodies specific for the interleukin 2 receptor (Queen et al., 1989 [17]) and also for antibodies specific for herpes simplex virus (HSV) (Co. et al., 1991 [19]). However, the reintroduction of murine residues into human frameworks (at least 9 for anti-interleukin 2 receptor antibodies, at least 9 and 7 for each of two anti-HSV antibodies) may increase the prospect of HAMA response to the framework region in the humanized antibody. Bruggemann et al., 1989 [20] have demonstrated that human V region frameworks are recognized as foreign in mouse, and so, conversely, murine modified human frameworks might give rise to an immune reaction in humans.

According to the above described two step approach in WO 90/07861 [18], Queen et al. outlined four criteria for designing humanized immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is usually homologous to the non-human donor immunoglobulin to be humanized, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the

framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanized immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, or each criteria may be applied singly or in any combination.

In addition, WO 90/07861 [18] details the preparation of a single CDR-grafted humanized antibody, a humanized antibody specificity for the p55 Tac protein of the IL-2 receptor, by employing the combination of all four criteria, as above, in designing this humanized antibody. The variable region frameworks of the human antibody EU (see, Kabat et al., 1991 [15]) were used as acceptor. In the resultant humanized antibody, the donor CDRs were as defined by Kabat et al., 1991 [15] and Wu et al., 1970 [16] and, in addition, the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanized anti-Tac antibody obtained was reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

Several other groups have demonstrated that Queen et al.'s approach of first choosing homologous frameworks followed by reintroduction of mouse residues may not be necessary to achieve humanized antibodies with similar binding affinities to the original mouse antibodies (Riechmann et al., 1988 [4]; Tempest et al., 1991 [5]; Verhoeyen, et al. 1991 [21]). Moreover, these groups

have used a different approach and have demonstrated that it is possible to utilize, as standard, the V region frameworks derived from NEWM and REI heavy and light chains respectively for CDR-grafting without radical introduction of mouse residues. However, the determination of which mouse residues should be introduced to produce antibodies with binding efficiencies similar to the original murine MAb can be difficult to predict, being largely empirical and not taught by available prior art. In the case of the humanized CAMPATH-IH antibody, the substitution of a phenylalanine for a serine residue at position 27 was the only substitution required to achieve a binding efficiency similar to that of the original murine antibody (Riechmann, et al., 1988 [4]; WO92/04381 [22]). In the case of a humanized (reshaped) antibody specific for respiratory syncytial virus (RSV) for the inhibition of RSV infection *in vivo*, substitution of a block of 3 residues adjacent to CDR3 in the CDR-grafted NEWM heavy chain was required to produce biological activity equivalent to the original mouse antibody (Tempest et al., 1991 [5]; WO 92/04381 [22]). The reshaped antibody in which only the mouse CDRs were transferred to the human framework showed poor binding for RSV. An advantage of using the Tempest et al., 1991 [5] approach to construct NEWM and REI based humanized antibodies is that the 3-dimensional structures of NEWM and REI variable regions are known from x-ray crystallography and thus specific interactions between CDRs and V region framework residues can be modelled.

Regardless of the approach taken, the examples of the initial humanized antibodies prepared to date have shown that it is not a straightforward process to obtain humanized antibodies with the characteristics, in particular, the binding affinity, as well as other desirable properties, of the original murine MAb from

which the humanized antibody is derived. Regardless of the approach to CDR grafting taken, it is often not sufficient merely to graft the CDRs from a donor Ig onto the framework regions of an acceptor Ig (see, e.g., Tempest et al., 1991 [5], Riechmann et al., 1988 [4], etc., cited herein). In a number of cases, it appears to be critical to alter residues in the framework regions of the acceptor antibody in order to obtain binding activity. However, even acknowledging that such framework changes may be necessary, it is not possible to predict, on the basis of the available prior art, which, if any, framework residues will need to be altered to obtain functional humanized recombinant antibodies of the desired specificity. Results thus far indicate that changes necessary to preserve specificity and/or affinity are for the most part unique to a given antibody and cannot be predicted based on the humanization of a different antibody.

In particular, the sets of residues in the framework region which are herein disclosed as being of critical importance to the activity of the recombinant humanized anti-VLA4 antibodies constructed in accordance with the teachings of the present invention do not generally coincide with residues previously identified as critical to the activity of other humanized antibodies and were not discovered based on the prior art.

C. Therapeutic Applications of Humanized Antibodies

To date, humanized recombinant antibodies have been developed mainly for therapeutic application in acute disease situations (Tempest, et al., 1991 [5]) or for diagnostic imaging (Verhoeyen, et al., 1991 [21]). Recently, clinical studies have begun with at least two humanized antibodies with NEWM and REI V region frameworks, CAMPATH-IH (Riechmann et al., 1988 [4]) and

humanized anti-placental alkaline phosphatase (PLAP) (Verhoeyen et al., 1991 [21]) and these studies have initially indicated the absence of any marked immune reaction to these antibodies. A course of treatment with CAMPATH-IH provided remission for two patients with non-Hodgkins lymphoma thus demonstrating efficacy in a chronic disease situation (Hale et al., 1988 [23]). In addition, the lack of immunogeneicity of CAMPATH-1H was demonstrated after daily treatment of the two patients for 30 and 43 days. Since good tolerance to humanized antibodies has been initially observed with CAMPATH-IH, treatment with humanized antibody holds promise for the prevention of acute disease and to treatment of diseases with low mortality.

D. The VCAM-VLA4 Adhesion Pathway and Antibodies to VLA4

Vascular endothelial cells constitute the lining of blood vessels and normally exhibit a low affinity for circulating leukocytes (Harlan, 1985 [24]). The release of cytokines at sites of inflammation, and in response to immune reactions, causes their activation and results in the increased expression of a host of surface antigens. (Collins et al., 1986 [25]; Pober et al., 1986 [26]; Bevilacqua et al., 1987 [27]; Leeuwenberg et al., 1989 [28]). These include the adhesion proteins ELAM-1, which binds neutrophils (Bevilacqua et al., 1989 [29], ICAM-1 which interacts with all leukocytes (Dustin et al., 1986 [30]; Pober et al. 1986, [26]; Boyd et al., 1988 [31]; Dustin and Springer, 1988 [32]), and VCAM-1 which binds lymphocytes (Osborn et al., 1989 [33]). These cytokine-induced adhesion molecules appear to play an important role in leukocyte recruitment to extravascular tissues.

The integrins are a group of cell-extracellular matrix and cell-cell adhesion receptors exhibiting an alpha-beta heterodimeric structure, with a widespread cell distribution and a high degree of conservation throughout evolution (Hynes, 1987 [34]; Marcantonio and

Hynes, 1988 [35]). The integrins have been subdivided into three major subgroups; the β_2 subfamily of integrins (LFA-1, Mac-1, and p150,95) is mostly involved in cell-cell interactions within the immune system (Kishimoto et al., 1989 [36]), whereas members of the β_1 and β_3 integrin subfamilies predominantly mediate cell attachment to the extracellular matrix (Hynes, 1987 [34]; Ruoslahti, 1988 [37]). In particular, the β_1 integrin family, also termed VLA proteins, includes at least six receptors that specifically interact with fibronectin, collagen, and/or laminin (Hemler, 1990 [38]). Within the VLA family, VLA4 is atypical because it is mostly restricted to lymphoid and myeloid cells (Hemler et al., 1987 [39]), and indirect evidence had suggested that it might be involved in various cell-cell interactions (Clayberger et al., 1987 [40]; Takada et al., 1989 [41]; Holtzmann et al., 1989 [42]; Bendarczyk and McIntyre, 1990 [43]). In addition, VLA4 has been shown to mediate T and B lymphocyte attachment to the heparin II binding fragment of human plasma fibronectin (FN) (Wayner et al., 1989 [44]).

VCAM-1, like ICAM-1, is a member of the immunoglobulin gene superfamily (Osborn et al., 1989 [33]). VCAM-1 and VLA4 were demonstrated to be a ligand-receptor pair that allows attachment of lymphocytes to activated endothelium by Elices et al., 1990 [45]. Thus, VLA4 represents a singular example of a β_1 integrin receptor participating in both cell-cell and cell-extracellular matrix adhesion functions by means of the defined ligands VCAM-1 and FN.

VCAM1 (also known as INCAM-110) was first identified as an adhesion molecule induced on endothelial cells by inflammatory cytokines (TNF and IL-1) and LPS (Rice et al., 1989 [46]; Osborn et al., 1989 [33]). Because VCAM1 binds to cells exhibiting the integrin VLA4 ($\alpha_4\beta_1$), including T and B lymphocytes, monocytes, and

eosinophils, but not neutrophils, it is thought to participate in recruitment of these cells from the bloodstream to areas of infection and inflammation (Elices et al, 1990 [45]; Osborn, 1990 [33]). The VCAM1/VLA4 adhesion pathway has been associated with a number of physiological and pathological processes. Although VLA4 is normally restricted to hematopoietic lineages, it is found on melanoma cell lines, and thus it has been suggested that VCAM1 may participate in metastasis of such tumors (Rice et al., 1989 [46]).

In vivo, VCAM1 is found on areas of arterial endothelium representing early atherosclerotic plaques in a rabbit model system (Cybulsky and Gimbrone, 1991 [47]). VCAM1 is also found on follicular dendritic cells in human lymph nodes (Freedman et al., 1990 [48]). It is also present on bone marrow stromal cells in the mouse (Miyake et al., 1991 [49]), thus VCAM1 appears to play a role in B-cell development.

The major form of VCAM1 in vivo on endothelial cells, has been referred to as VCAM-7D, and has seven Ig homology units or domains; domains 4, 5 and 6 are similar in amino acid sequence to domains 1, 2 and 3, respectively, suggesting an intergenic duplication event in the evolutionary history of the gene (Osborn et al., 1989 [33]; Polte et al. 1990 [50]; Hession et al., 1991 [51]; Osborn and Benjamin, U.S. Ser. No. 07/821,712 filed September 30, 1991, [52]). A 6-domain form (referred to as VCAM-6D herein) is generated by alternative splicing, in which the fourth domain is deleted (Osborn et al., 1989 [33]; Hession et al. 1991 [51], Cybulsky et al., 1991 [47]; Osborn and Benjamin, U.S. Ser. No. 07/821,712 filed September 30, 1991 [52]). The VCAM-6D, was the first sequenced of these alternate forms, however, later in vivo studies showed that the VCAM-7D form was dominant in vivo. The biological significance of the alternate splicing is not known, however as shown by Osborn and

Benjamin, U.S. Ser. No. 07/821,712 filed September 30, 1991 [52], VCAM-6D can bind VLA4-expressing cells and thus clearly has potential functionality in vivo.

The apparent involvement of the VCAM1/VLA4 adhesion pathway in infection, inflammation and possibly atherosclerosis has led to continuing intensive research to understand the mechanisms of cell-cell adhesion on a molecular level and has led investigators to propose intervention in this adhesion pathway as a treatment for diseases, particularly inflammation (Osborn et al., 1989 [33]). One method of intervention in this pathway could involve the use of anti-VLA4 antibodies.

Monoclonal antibodies that inhibit VCAM1 binding to VLA4 are known. For example, anti-VLA4 MAbs HP2/1 and HP1/3 have been shown to block attachment of VLA4-expressing Ramos cells to human umbilical vein cells and VCAM1-transfected COS cells (Elices et al., 1990 [45]). Also, anti-VCAM1 antibodies such as the monoclonal antibody 4B9 (Carlos et al., 1990 [53]) have been shown to inhibit adhesion of Ramos (B-cell-like), Jurkat (T-cell-like) and HL60 (granulocyte-like) cells to COS cells transfected to express VCAM-6D and VCAM-7D (Hession et al., 1991 [51]).

The monoclonal antibodies to VLA4 that have been described to date fall into several categories based on epitope mapping studies (Pulido, et al., 1991 [54]). Importantly one particular group of antibodies, to epitope "B", are effective blockers of all VLA4-dependent adhesive functions (Pulido et al., 1991, [54]). The preparation of such monoclonal antibodies to epitope B of VLA 4, including, for example the HP1/2 MAb, have been described by Sanchez-Madrid et al., 1986, [55]. Antibodies having similar specificity and having high binding affinities to VLA4 comparable to that of HP1/2, would be particularly promising candidates for the

preparation of humanized recombinant anti-VLA4 antibodies useful as assay reagents, diagnostics and therapeutics.

As stated above, inflammatory leukocytes are recruited to sites of inflammation by cell adhesion molecules that are expressed on the surface of endothelial cells and which act as receptors for leukocyte surface proteins or protein complexes. In particular, eosinophils have recently been found to participate in three distinct cell adhesion pathways to vascular endothelium, binding to cells expressing intercellular adhesion molecule-1 (ICAM-1), endothelial cell adhesion molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (Weller et al., 1991 [56]; Walsh et al., 1991 [57]; Bochner et al., 1991 [58]; and Dobrina et al., 1991 [59]). That eosinophils express VLA4 differentiates them from other inflammatory cells such as neutrophils, which bind to ELAM-1 and ICAM-1 but not VCAM-1.

The VLA4-mediated adhesion pathway has been investigated in an asthma model to examine the possible role of VLA4 in leukocyte recruitment to inflamed lung tissue (Lobb, U.S. Ser. No. 07/821,768 filed January 13, 1992 [60]). Administering anti-VLA4 antibody inhibited both the late phase response and airway hyperresponsiveness in allergic sheep. Surprisingly, administration of anti-VLA4 led to a reduction in the number of both neutrophils and eosinophils in the lung at 4 hours after allergen challenge, even though both cells have alternate adhesion pathways by which they can be recruited to lung tissues. Also surprisingly, inhibition of hyperresponsiveness in the treated sheep was observed which continued to 1 week, even though infiltration of leukocytes, including neutrophils and eosinophils, was not significantly reduced over time.

The VLA4-mediated adhesion model has also been investigated in a primate model of inflammatory bowel

disease (IBD) (Lobb, U.S. Ser. No, 07/835,139 filed February 12, 1992 [61]). The administration of anti-VLA4 antibody surprisingly and significantly reduced acute inflammation in that model, which is comparable to ulcerative colitis in humans.

More recently, anti-VLA4 antibodies have been used in methods for the peripheralizing of $CD34^+$ cells, including hematopoietic stem cells as described in Papyannopoulou, U.S. Ser. No. 07/977,702, filed November 13, 1992 [62].

Thus, anti-VLA4 antibodies having certain epitopic specificities and certain binding affinities may be therapeutically useful in a variety of inflammatory conditions, including asthma and IBD. In particular, humanized recombinant versions of such anti-VLA4 antibodies, if they could be constructed, might be especially useful for administration in humans. Such humanized antibodies would have the desired potency and specificity, while avoiding or minimizing an immunological response which would render the antibody ineffective and/or give rise to undesirable side effects.

SUMMARY OF THE INVENTION

The present invention provides a method of constructing a recombinant anti-VLA4 antibody molecule. Specifically, recombinant antibodies according to the present invention comprise the antigen binding regions derived from the heavy and/or light chain variable regions of an anti-VLA4 antibody.

The present invention provides a method for the construction of humanized recombinant antibody molecule using as a first step CDR grafting or "reshaping" technology. Specifically, the humanized antibodies according to the present invention have specificity for VLA4 and have an antigen binding site wherein at least one or more of the complementarity determining regions (CDRs) of the variable domains are derived from a donor non-human anti-VLA4 antibody, and in which there may or may not have been minimal alteration of the acceptor antibody heavy and/or light variable framework region in order to retain donor antibody binding specificity. Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise the CDRs corresponding to positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). These residue designations are numbered according to the Kabat numbering (Kabat et al., 1991 [15]). Thus, the residue/position designations do not always correspond directly with the linear numbering of the amino acid residues shown in the sequence listing. In the case of the humanized V_K sequence disclosed herein, the Kabat numbering does actually correspond to the linear numbering of amino acid residues shown in the sequence listing. In contrast, in the case of the humanized V_H sequences disclosed herein, the Kabat numbering does not correspond to the linear numbering of

amino acid residues shown in the sequence listing (e.g., for the humanized V_H regions disclosed in the sequence listing, CDR2 = 50-66, CDR3 = 99-110).

The invention further provides the recombinant and humanized anti-VLA4 antibodies which may be detectably labelled.

The invention additionally provides a recombinant DNA molecule capable of expressing the recombinant and humanized anti-VLA4 antibodies of the present invention.

The invention further provides host cells capable of producing the recombinant and humanized anti-VLA4 antibodies of the present invention.

The invention additionally relates to diagnostic and therapeutic uses for the recombinant and humanized anti-VLA4 antibodies of the present invention.

The invention further provides a method for treating inflammation resulting from a response of the specific defense system in a mammalian subject, including humans, which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the inflammation wherein the anti-inflammatory agent is a recombinant and humanized anti-VLA4 antibody of the present invention.

The invention further provides a method for treating non-specific inflammation in a mammalian subject, including humans using the recombinant and humanized anti-VLA4 antibodies.

The invention further concerns the embodiment of the above-described methods wherein the recombinant and humanized anti-VLA4 antibodies of the present invention are derived from the murine monoclonal antibody HP1/2.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The technology for producing monoclonal antibodies is well known. Briefly, an immortal cell line (typically myeloma cells) is fused to lymphocytes (typically splenocytes) from a mammal immunized with whole cells expressing a given antigen, e.g., VLA4, and the culture supernatants of the resulting hybridoma cells are screened for antibodies against the antigen (see, generally, Kohler et al., 1975 [1]).

Immunization may be accomplished using standard procedures. The unit dose and immunization regimen depend on the species of mammal immunized, its immune status, the body weight of the mammal, etc. Typically, the immunized mammals are bled and the serum from each blood sample is assayed for particular antibodies using appropriate screening assays. For example, anti-VLA4 antibodies may be identified by immunoprecipitation of ^{125}I-labeled cell lysates from VLA4-expressing cells (see, Sanchez-Madrid et al., 1986 [55] and Hemler et al., 1987 [39]). Anti-VLA-4 antibodies may also be identified by flow cytometry, e.g., by measuring fluorescent staining of Ramos cells incubated with an antibody believed to recognize VLA4 (see, Elices et al., 1990 [45]). The lymphocytes used in the production of hybridoma cells typically are isolated from immunized mammals whose sera have already tested positive for the presence of anti-VLA4 antibodies using such screening assays.

Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium").

Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using 1500 molecular weight polyethylene glycol ("PEG 1500"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridomas producing a desired antibody are detected by screening the hybridoma culture supernatants. For example, hybridomas prepared to produce anti-VLA4 antibodies may be screened by testing the hybridoma culture supernatant for secreted antibodies having the ability to bind to a recombinant α_4-subunit-expressing cell line, such as transfected K-562 cells (see, e.g., Elices et al., 1990 [45]).

To produce anti VLA4-antibodies, hybridoma cells that tested positive in such screening assays are cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. Tissue culture techniques and culture media suitable for hybridoma cells are well known. The conditioned hybridoma culture supernatant may be collected and the anti-VLA4 antibodies optionally further purified by well-known methods.

Alternatively, the desired antibody may be produced by injecting the hybridoma cells into the peritoneal cavity of an unimmunized mouse. The hybridoma cells proliferate in the peritoneal cavity, secreting the antibody which accumulates as ascites fluid. The antibody may be harvested by withdrawing the ascites fluid from the peritoneal cavity with a syringe.

Several anti-VLA4 monoclonal antibodies have been previously described (see, e.g., Sanchez-Madrid et al., 1986 [55]; Hemler et al., 1987 [39]; Pulido et al., 1991 [54]). HP1/2, for example, is one such murine monoclonal antibody which recognizes VLA4. VLA4 acts as a leukocyte

receptor for plasma fibronectin and VCAM-1. Other monoclonal antibodies, such as HP2/1, HP2/4, L25 and P4C2, have been described that also recognize VLA4.

Recombinant antibodies have been constructed and are described herein in which the CDRs of the variable domains of both heavy and light chains were derived from the murine HP1/2 sequence. Preferred starting materials for constructing recombinant humanized antibodies according to the present invention are anti-VLA4 antibodies, such as HP1/2, that block the interaction of VLA4 with both VCAM1 and fibronectin. Particularly preferred are those antibodies, such as HP1/2, which in addition, do not cause cell aggregation. Some anti-VLA4 blocking antibodies have been observed to cause such aggregation. The HP1/2 MAb (Sanchez-Madrid et al., 1986 [55]) is a particularly excellent candidate for humanization since it has an extremely high potency, blocks VLA4 interaction with both VCAM1 and fibronectin, but does not cause cell aggregation, and has the specificity for epitope B on VLA4. In the initial experiments, V_H and V_K DNA were isolated and cloned from an HP1/2-producing hybridoma cell line. The variable domain frameworks and constant domains for humanization were initially derived from human antibody sequences.

The three CDRs that lie on both heavy and light chains are composed of those residues which structural studies have shown to be involved in antigen binding. Theoretically, if the CDRs of the murine HP1/2 antibody were grafted onto human frameworks to form a CDR-grafted variable domain, and this variable domain were attached to human constant domains, the resulting CDR-grafted antibody would essentially be a human antibody with the specificity of murine HP1/2 to bind human VLA4. Given the highly "human" nature of this antibody, it would be expected to be far less immunogenic than murine HP1/2 when administered to patients.

However, following testing for antigen binding of a CDR-grafted HP1/2 antibody in which only the CDRs were grafted onto the human framework, it was shown that this did not produce a CDR-grafted antibody having reasonable affinity for the VLA4 antigen. It was therefore decided that additional residues adjacent to some of the CDRs and critical framework residues needed to be substituted from the human to the corresponding murine HP1/2 residues in order to generate an antibody with binding affinity in the range of 10% to 100% of the binding affinity of the murine HP1/2 MAb. Empirically, changes of one or more residues in the framework regions of V_H and V_K were made to prepare antibodies of the desired specificity and potency, but without making so many changes in the human framework so as to compromise the essentially human nature of the humanized V_H and V_K region sequences.

Furthermore, VLA4-binding fragments may be prepared from the recombinant anti-VLA4 antibodies described herein, such as Fab, Fab', $F(ab')_2$, and F(v) fragments; heavy chain monomers or dimers; light chain monomers or dimers; and dimers consisting of one heavy chain and one light chain are also contemplated herein. Such antibody fragments may be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, or via recombinant DNA techniques, e.g., by using host cells transformed with truncated heavy and/or light chain genes. Heavy and light chain monomers may similarly be produced by treating an intact antibody with a reducing agent such as dithiothreitol or β-mercaptoethanol or by using host cells transformed with DNA encoding either the desired heavy chain or light chain or both.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not intended to be limiting in nature. In the following examples, the necessary restriction enzymes, plasmids, and other reagents and materials may be obtained from commercial sources and cloning, ligation and other recombinant DNA methodology may be performed by procedures well-known in the art.

Example 1

Isolation of DNA Sequences Encoding Murine Anti-VLA4 Variable Regions

A. Isolation of the HP1/2 heavy and light chain cDNA

To design a humanized recombinant antibody with specificity for VLA4, it was first necessary to determine the sequence of the variable domain of the murine HP1/2 heavy and light chains. The sequence was determined from heavy and light chain cDNA that had been synthesized from cytoplasmic RNA according to methods referenced in Tempest et al., 1991 [5].

1. Cells and RNA isolation

Cytoplasmic RNA (~200 μg) was prepared by the method of Favaloro et al., 1980 [63], from a semi-confluent 150cm^2 flask of HP1/2-producing hybridoma cells (about 5 X 10^5 logarithmic phase cells). The cells were pelleted and the supernatant was assayed for the presence of antibody by a solid phase ELISA using an Inno-Lia mouse monoclonal antibody isotyping kit (Innogenetics, Antwerp, Belgium) using both the kappa conjugate and the lambda conjugate. The antibody was confirmed to be IgG1/κ by this method.

2. cDNA Synthesis

cDNAs were synthesized from the HP1/2 RNA via reverse transcription initiated from primers based on the 5′ end of either the murine IgG1 CH_1 or the murine kappa constant domains using approximately 5 μg RNA and 25 pmol primer in reverse transcriptase buffer containing 1 μl/50 μl Pharmacia (Milton Keynes, United Kingdom) RNA Guard™ and 250 micromolar dNTPs. The sequence of these primers, CG1FOR and CK2FOR are shown as SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The mixture was heated to 70°C, then allowed to cool slowly to room temperature. Then, 100 units/50 μl MMLV reverse transcriptase (Life Technologies, Paisley, United Kingdom) was added and the reaction was allowed to proceed at 42°C for one hour.

3. Amplification of V_H and V_K cDNA

Polymerase chain reaction (PCR) of murine MAb variable regions can be achieved using a variety of procedures, for example, anchored PCR or primers based on conserved sequences (see, e.g., Orlandi et al., 1989 [64]). Orlandi et al. [64], Huse et al., 1989 [65] and Jones and Bendig, 1991 [66], have described some variable region primers. We have been unsuccessful, however, in using a number of such primers, particularly those for the light chain PCR of HP1/2 derived V_K sequences.

HP1/2 Ig V_H and V_K cDNAs were amplified by PCR as described by Saiki et al., 1988 [67] and Orlandi et al., 1989 [64]. Reactions were carried out using 2.5 units/50 μl Amplitaq™ polymerase (Perkin Elmer Cetus, Norwalk, CT) in 25 cycles of 94°C for 30 seconds followed by 55°C for 30 seconds and 75°C for 45 seconds. The final cycle was followed by five minute incubation at 75°C. The same 3′ oligonucleotides used for cDNA synthesis were used in conjunction with appropriate 5′ oligonucleotides based on consensus sequences of relatively conserved regions at the 5′ end of each V region. V_H cDNA was successfully amplified using the primers VH1BACK [SEQ ID NO: 3] and CG1FOR [SEQ ID NO: 1] and yielded an amplification product of approximately 400 bp. V_K cDNA was successfully amplified using the primers VK5BACK [SEQ ID NO: 4] and CK2FOR [SEQ ID NO: 2] and yielded an amplification product of approximately 380 bp.

4. Cloning and Sequencing V_H DNA

The primers used for the amplification of V_H DNA, contain the restriction enzyme sites PstI and HindIII which facilitate cloning into sequencing vectors. The general cloning and ligation methodology was as described in Molecular Cloning, A Laboratory Manual 1982, [68]. The

amplified DNA was digested with PstI to check for internal PstI sites and an internal PstI site was found. Therefore, the V_H DNA was cloned as PstI-PstI and PstI-HindIII fragments into M13mp18 and 19. The resulting collection of clones from two independent cDNA preparations were sequenced by the dideoxy method (Sanger, et al., 1977, [69] using Sequenase™ (United States Biochemicals, Cleveland, Ohio, USA). The sequence of a region of ~100-250 bp was determined from each of 25 clones. Out of more than 4000 nucleotides sequenced, there were three PCR-induced transition mutation in three separate clones. The HP1/2 V_H DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 5 and SEQ ID NO: 6, respectively. It should be noted that the first eight amino acids are dictated by the 5′ primer used in the PCR. Computer-assisted comparisons indicate that HP1/2 V_H [SEQ ID NOS: 5 and 6] is a member of family IIC (Kabat et al., 1991, [15]. A comparison between HP1/2 V_H [SEQ ID NOS: 5 and 6] and a consensus sequence of family IIC revealed that the only unusual residues are at amino acid positions 80, 98 and 121 (79, 94 and 121 in Kabat numbering). Although Tyr 80 is invariant in subgroup IIC other sequenced murine V_H regions have other aromatic amino acids at this position although none have Trp. The majority of human and murine V_Hs have an arginine residue at Kabat position 94. The presence of Asp 94 in HP1/2 V_H is extremely rare; there is only one reported example of a negatively charged residue at this position. Proline at Kabat position 113 is also unusual but is unlikely to be important in the conformation of the CDRs because of its distance from them. The amino acids making up CDR1 have been found in three other sequenced murine V_H regions. However, CDR2 and CDR3 are unique to HP1/2 and are not found in any other reported murine V_H.

5. Cloning and Sequencing V_K DNA

The primers used for the amplification of V_K DNA contain restriction sites for the enzymes EcoRI and HindIII. The PCR products obtained using primers VK1BACK [SEQ ID NO: 7], VK5BACK [SEQ ID NO: 4] and VK7BACK [SEQ ID NO: 8] were purified and cloned into M13. Authentic kappa sequences were obtained only with VK5BACK [SEQ ID NO: 4]. The sequence of a region of ~200-350 bp was determined by the dideoxy method (Sanger et al., 1977, [69] using Sequenase™ (United States Biochemicals, Cleveland, Ohio, USA) from each of ten clones from two independent cDNA preparations. Out of more than 2 kb sequenced, there were only two clones which each contained one PCR-induced transition mutation.

The HP1/2 V_K DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 9 and SEQ ID NO: 10, respectively. The first four amino acids are dictated by the 5′ PCR primer but the rest of the sequence is in total agreement with partial protein sequence data. HP1/2 V_K is a member of Kabat family V (Kabat et al., 1991 [15]) and has no unusual residues. The amino acids of CDR1 and CDR3 are unique. The amino acids making up CDR2 have been reported in one other murine V_K.

Example 2

Design of a CDR-grafted Anti-VLA4 Antibody

To design a CDR-grafted anti-VLA4 antibody, it was necessary to determine which residues of murine HP1/2 comprise the CDRs of the light and heavy chains.

Three regions of hypervariability amid the less variable framework sequences are found on both light and heavy chains (Wu and Kabat, 1970 [16]; Kabat et al., 1991 [15]). In most cases these hypervariable regions correspond to, but may extend beyond, the CDR. The amino acid sequences of the murine HP1/2 V_H and V_K chains are

set forth in SEQ ID NO: 6 and SEQ ID NO: 10, respectively. CDRs of murine HP1/2 were elucidated in accordance with Kabat et al., 1991 [15] by alignment with other V_H and V_K sequences. The CDRs of murine HP1/2 V_H were identified and correspond to the residues identified in the humanized V_H sequences disclosed herein as follows:

CDR1	AA_{31}-AA_{35}
CDR2	AA_{50}-AA_{66}
CDR3	AA_{99}-AA_{110}

These correspond to AA_{31}-AA_{35}, AA_{50}-AA_{65}, and AA_{95}-AA_{102}, respectively, in Kabat numbering. The CDRs of murine HP1/2 V_K were identified and correspond to the residues identified in the humanized V_K sequences disclosed herein as follows:

CDR1	AA_{24}-AA_{34}
CDR2	AA_{50}-AA_{56}
CDR3	AA_{89}-AA_{97}

These correspond to the same numbered amino acids in Kabat numbering. Thus, only the boundaries of the V_K, but not V_H, CDRs corresponded to the Kabat CDR residues. The human frameworks chosen to accept the HP1/2 CDRs were NEWM and REI for the heavy and light chains respectively. The NEWM and the REI sequences have been published in Kabat et al., 1991 [15].

An initial stage of the humanization process may comprise the basic CDR grafting with a minimal framework change that might be predicted from the literature. For example, in Riechmann et al., 1988 [4], the MAb CAMPATH-1H was successfully humanized using direct CDR grafting with only one framework change necessary to obtain an antibody with a binding efficiency similar to that of the original murine antibody. This framework change was the substitution of a Phe for a Ser at position 27. However, using the same humanization strategy by CDR grafting and the single framework change discovered by Riechmann et

al., 1988 [4] for the preparation of humanized antibodies having other specificities did not yield antibodies with affinities comparable to the murine antibodies from which they were derived. In such cases, the humanization process must necessarily include additional empirical changes to achieve the desired specificity and potency. Such changes may be related to the unique structure and sequence of the starting murine antibody but are not predictable based upon other antibodies of different specificity and sequence. For example, analysis of the murine V_H amino acid sequence from HP1/2 as set forth in SEQ ID NO: 6 as compared with the other known sequences indicated that residues 79, 94 and 113 (Kabat numbering) were unusual. Of these, only Asp 94 is likely to be important in CDR conformation. Most V_H regions that have been sequenced have an arginine at this position which is able to form a salt bridge with a relatively conserved Asp 101 in CDR3. Because NEWM has an Arg 94 and V_H CDR3 of HP1/2 has an Asp 101, there remains the possibility that a salt bridge would form which would not normally occur. The presence of a negatively charged residue at position 94 is very unusual and therefore it was decided to include the Asp 94 into the putative humanized V_H.

A chimeric (murine V/human IgG1/κ) HP1/2 antibody may be useful, but not a necessary, intermediate in the initial stages of preparing a CDR grafted construct because (i) its antigen-binding ability may indicate that the correct V regions have been cloned; and (ii) it may act as a useful control in assays of the various humanized antibodies prepared in accordance with the present invention.

For V_H, an M13 clone containing full-length HP1/2 V_H was amplified using VH1BACK [SEQ ID NO: 3] and VH1FOR [SEQ ID NO: 11] which contain PstI and BstEII sites respectively at the 5′ and 3′ ends of the V_H domain. The amplified DNA was cut with BstEII and partially cut with

PstI, full-length DNA purified and cloned into M13VHPCR1 (Orlandi et al., 1989 [64]) which had been cut with PstI and BstEII. For V_K an M13 clone containing full-length HP1/2 V_K was amplified using VK3BACK [SEQ ID NO: 12] and VK1FOR [SEQ ID NO: 13] to introduce PvuII and BglII sites respectively at the 5′ and 3′ ends of the V_K domain. The amplified DNA was cut with PvuII and BglII and cloned into M13VKPCR1 (Orlandi et al., 1989 [64]) which had been cut with PvuII and BclI.

In sum, the 5′ primers used for the amplification of the murine V_H and V_K regions contain convenient restriction sites for cloning into our expression vectors. The 3′ primers used in the PCRs were from the constant regions. Restriction sites at the 3′ end of the variable regions were introduced into cloned murine variable region genes with PCR primers which introduced BstII or BglII sites in the heavy and light (kappa) variable regions, respectively. Additionally, the V_H primer changed Pro 113 to Ser.

The murine V_H and V_K DNAs were cloned into vectors containing the gpt and hygromycin resistance genes respectively, such as pSVgpt and pSVhyg as described by Orlandi, et al. [64], and appropriate human IgG1, IgG4 or κ constant regions were added, for example, as described by Takahashi et al., 1982 [70], Flanagan and Rabbitts, 1982 [71], and Hieter et al., 1980 [72], respectively. The vectors were cotransfected into the rat myeloma YB2/0 and mycophenolic acid resistant clones screened by ELISA for secretion of chimeric IgG/κ antibody. The YB2/0 cell line was described by Kilmartin et al., 1982 [73] and is available from the American Type Culture Collection (ATCC, Rockville, MD). ELISA positive clones were expanded and antibody purified from culture medium by protein A affinity chromatography. The chimeric antibody purified from the transfected cells was assayed for anti-

VLA4 antibody activity as described in Example 7 and was found to be equipotent with the murine HP1/2 antibody.

Example 3

Transplantation of CDR Sequences and Mutagenesis of Selected Framework Residues

Transplantation of the CDRs into human frameworks was performed using M13 mutagenesis vectors. The human frameworks chosen to accept the CDR sequences outlined in Example 2 were derived from NEWM for V_H and REI for V_K, each in an M13 mutagenesis vector. The M13 mutagenesis vectors used for V_H and V_K, were M13VHPCR1 and M13VKPCR2, respectively. M13VKPCR2 is identical to M13VKPCR1 as described by Orlandi et al., 1989 [64], except for a single amino acid change from valine (GTG) to glutamine (GAA) in framework 4 of the REI V_K coding sequence. M13VHPCR1 described by Orlandi et al., 1989 [64] is M13 that contains the coding sequence for a V_H region that is an NEWM framework sequence with CDRs derived from an anti-hapten (4-hydroxy-3-nitrophenyl acetyl caproic acid) antibody; the irrelevant V_H CDRs are replaced by site-directed mutagenesis with the CDRs derived from HP1/2 V_H as described below. The V_H region sequence (DNA and amino acid) encoded by M13VHPCR1 is shown as SEQ ID NOS: 14 and 15. M13VKPCR2, like M13VKPCR1 described by Orlandi et al. [64], is M13 that contains the coding sequence for a V_K region that is N-terminal modified REI framework sequence with CDRs derived from an anti-lysozyme antibody; these irrelevant V_K CDRs are replaced by site-directed mutagenesis with the CDRs derived from HP1/2 V_K as described below. The V_K region sequence (DNA and amino acid) encoded by M13PCR2 is shown as SEQ ID NOS: 16 and 17.

Synthetic oligonucleotides were synthesized containing the HP1/2-derived V_H and V_K CDRs flanked by short sequences drawn from NEWM and REI frameworks,

respectively, and grafted into the human frameworks by oligonucleotide site-directed mutagenesis as follows. For CDR grafting into the human V_H framework, mutagenizing oligonucleotides 598 [SEQ ID NO: 18], 599 [SEQ ID NO: 19] and 600 [SEQ ID NO: 20] were used. For CDR grafting into the human V_K framework, the mutagenizing oligonucleotides were 605 [SEQ ID NO: 21], 606 [SEQ ID NO: 22] and 607 [SEQ ID NO: 23]. To 5 μg of V_H or V_K single-stranded DNA in M13 was added a 2-fold molar excess of each of the three V_H or V_K phosphorylated oligonucleotides together with flanking primers based on M13 sequences, oligo 10 [SEQ ID NO: 24] for V_H and oligo 385 [SEQ ID NO: 25] for V_K. Primers were annealed to the template by heating to 70°C and slowly cooling to 37°C. The annealed DNA was extended and ligated with 2.5 U T7 DNA polymerase (United States Biochemicals) and 1 U T4 DNA ligase (Life Technologies) in 10 mM Tris HCl pH 8.0, 5 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 250 μM dNTPs in a reaction volume of 50 μl at 16°C for 1-2 hours.

The newly extended mutagenic strand was preferentially amplified using 1 U Vent DNA polymerase (New England Biolabs) and 25 pmol oligo 11 [SEQ ID NO: 26] or oligo 391 [SEQ ID NO: 27] (for V_H or V_K, respectively) in 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris HCl pH 8.8, 2 mM $MgSO_4$, 0.1% Triton X-100, 25 μM dNTPs in a reaction volume of 50 μl and subjecting the sample to 30 cycles of 94°, 30s; 50°, 30s; 75°, 90s.

A normal PCR was then performed by adding 25 pmol oligo 10 [SEQ ID NO: 24] (for V_H) or oligo 385 [SEQ ID NO: 25] (for V_K) with 10 thermal cycles. The product DNAs were digested with HindIII and BamHI and cloned into M13mp19. Single-stranded DNA was prepared from individual plaques, sequenced and triple mutants were identified.

The resulting Stage 1 V_H construct with the DNA sequence and its translated product set forth in SEQ ID

NO: 28 and SEQ ID NO: 29, respectively. In addition to the CDR grafting, the Stage 1 V_H construct contained selected framework changes. Just prior to CDR1, a block of sequences was changed to the murine residues Phe 27, Asn 28, Ile 29 and Lys 30 [compare AA_{27}-AA_{30} of SEQ ID NO: 29 with that of murine V_H sequence [SEQ ID NO: 6]]. This included Phe-27 as substituted in the humanization of the rat CAMPATH1-H antibody (Riechmann et al., 1988 [4]), but then also substitutes the next three residues found in the murine sequence. Although these four residues are not nominally included in CDR1 (i.e., are not hypervariable in the Kabat sense), structurally they are a part of the CDR1 loop (i.e., structural loop residues), and therefore included empirically as part of CDR1. In addition, the change from Arg to Asp at residue 94 was made based on the rationale discussed in Example 2. An alignment of the CDR-grafted Stage 1 framework sequences as compared with the NEWM framework is shown in Table I. The resulting VK1 (DQL) construct with the DNA sequence and its translated product are set forth in SEQ ID NO: 30 and SEQ ID NO: 31, respectively. An alignment of the CDR-grafted VK1 (DQL) framework sequences as compared with the REI framework is shown in Table II.

The CDR replaced V_H (Stage 1) and V_K (VK1) genes were cloned in expression vectors according to Orlandi, et al., 1989 [64] to yield the plasmids termed pHuVHHuIgG1, pHuVHHuIgG4 and pHuVKHuCK. For pHuVHHuIgG1 and pHuVHHuIgG4, the Stage 1 V_H gene together with the Ig heavy chain promoter, appropriate splice sites and signal peptide sequences were excised from the M13 mutagenesis vector by digestion with HindIII and BamHI, and cloned into an expression vector such as pSVgpt as described by Orlandi et al. [64], containing the murine Ig heavy chain enhancer, the SV40 promoter, the gpt gene for selection in mammalian cells and genes for replication and selection in E. coli. A human IgG1 constant region as

described in Takahashi et al., 1982 [70] was then added as a BamHI fragment. Alternatively, a human IgG4 construct region as described by Flanagan and Rabbitts, 1982 [71] is added. The construction of the pHuVKHuCK plasmid, using an expression vector such as pSVhyg as described by Orlandi et al. [64], was essentially the same as that of the heavy chain expression vector except that the gpt gene for selection was replaced by the hygromycin resistance gene (hyg) and a human kappa chain constant region as described by Hieter, 1980, [72] was added. The vectors were cotransfected into the rat myeloma YB2/0 and mycophenolic acid resistant clones screened by ELISA for secretion of human IgG/κ antibody. The YB2/0 cell line was described by Kilmartin et al., 1982 [73] and is available from the American Type Culture Collection (ATCC, Rockville, MD). ELISA positive clones were expanded and antibody purified from culture medium by protein A affinity chromatography. The transfected cells are assayed for anti-VLA4 antibody activity as described in Example 7.

Example 4

Modification of a CDR grafted Antibody

Beyond the stages of design and preparation to yield anti-VLA4 antibodies as described above in Examples 2 and 3, additional stages of empirical modifications were used to successfully prepare humanized recombinant anti-VLA4 antibodies. The Stage 1 modifications as described in Example 3 were based on our analysis of primary sequence and experience in attempting to successfully humanize antibodies. The next modifications, designated as Stage 2, were empirical, based in part on our analysis of 3D modelling data. For the V_H region, further modifications, designated Stage 3, were so-called "scanning" modifications empirically made to correct any remaining defects in affinities or other antibody

properties. The modifications that were made in these several stages were empirical changes of various blocks of amino acids with the goal of optimizing the affinity and other desired properties of humanized anti-VLA4 antibodies. Not every modification made during the various stages resulted in antibodies with desired properties.

1. Additional heavy chain modifications

a. Stage 2 Modification

An additional empirical change in the V_H framework was made with the use of computer modelling, to generate a Stage 2 construct with the DNA sequence and its translated product set forth in SEQ ID NO: 32 and SEQ ID NO: 33, respectively. Using computer modelling of the Stage 1 V_H region, we determined to make a single change in the framework for Stage 2, namely a substitution of a Ser for Lys at position 75 (Kabat numbering), that is position 76 in SEQ ID NO: 33. This determination was in part based on the possibility that Lys-75 might project into CDR1 and alter its conformation. The M13 vector containing the Stage 1 CDR grafted HuVH, as described in Example 3, was used as template for two-step PCR-directed mutagenesis using the overlap/extension method as described by Ho et al., 1989 [74]. In the first step, two separate PCRs were set up, one with an end primer, oligo 10, [SEQ ID NO: 24] and a primer containing the desired mutation, 684 [SEQ ID NO: 34], and the other with the opposite end primer, oligo 11 [SEQ ID NO: 26], and a primer, 683 [SEQ ID NO: 35], that is complementary to the first mutagenic primer. The amplification products of this first pair of PCRs were then mixed together and a second PCR step was carried out using only the end primers oligos 10 and 11, SEQ ID NO: 24 and SEQ ID NO: 26, respectively. The mutagenized amplification product of this PCR was then cloned into M13mp19 and

sequenced, and a mutant bearing the Lys to Ser change (Stage 2 or "S mutant") was identified.

This turned out to be a critical change in the humanized heavy chain derived from HP1/2 (see Example 7). However, this critical change in the preparation of humanized recombinant anti-VLA4 antibodies according to the present invention was not similarly critical in the preparation of other humanized antibodies. Specifically, using the same rationalization and analysis as outlined above, a change in that position was not found to be a beneficial change in the humanization of antibodies of 2 different specificities. An alignment of the CDR-grafted Stage 2 framework sequences as compared with the NEWM, as well as Stage 1 sequences, is shown in Table I.

b. Stage 3 Modifications

Additional empirical changes were made as Stage 3 constructs. In Stage 3, a series of 5 different block changes of amino acids, for largely empirical reasons, were made to try to improve potency. These constructs are designated STAW, KAITAS, SSE, KRS, and AS. All contain the position 75 Ser (Kabat numbering) changed in Stage 2 [position 76 of SEQ ID NO: 35], with other changes as noted. Each of these constructs was prepared by two-step PCR directed mutagenesis using the overlap/extension method of Ho et al., 1989 [74], as described for the Stage 2 Ser mutant, above. For STAW, the additional changes were Gln to Thr at position 77, Phe to Ala at position 78 and Ser to Trp at position 79 (Kabat numbering). These changes were accomplished using end primers, oligos 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] in conjunction with mutagenizing primers 713 [SEQ ID NO: 36] and 716 [SEQ ID NO: 37]. The STAW V_H DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 38 and SEQ ID NO: 39, respectively. KAITAS was prepared with additional changes of Arg to Lys

(position 66), Val to Ala (67), Met to Ile (69), Leu to Thr (70) and Val to Ala (71) (Kabat numbering), using oligos 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] in conjunction with oligos 706 [SEQ ID NO: 40] and 707 [SEQ ID NO: 41]. The KAITAS V_H DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 42 and SEQ ID NO: 43, respectively. SSE had additional changes of Ala to Ser (84) and Ala to Glu (85) (Kabat numbering), effected by oligos 10 and 11 with oligos 768 [SEQ ID NO: 44] and 769 [SEQ ID NO: 45]. The SSE V_H DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 46 and SEQ ID NO: 47, respectively. KRS had additional changes of Arg to Lys (38) and Pro to Arg (40) (Kabat numbering), from oligos 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] with oligos 704 [SEQ ID NO: 48] and 705 [SEQ ID NO: 49]. The KRS V_H DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 50 and SEQ ID NO: 51, respectively. AS had additional change Val to Ala at position 24 (Kabat numbering) from oligos 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] with oligos 745 [SEQ ID NO: 52] and 746 [SEQ ID NO: 53]. The AS V_H DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 54 and SEQ ID NO: 55, respectively. An alignment of the CDR-grafted Stage 3 framework sequences with the NEWM, Stage 0 (see below), Stage 1, and Stage 2 sequences is shown in Table I. Importantly, as shown in Example 7, the potency of STAW and AS humanized antibodies were improved, while KAITAS and KRS humanized antibodies were not of better potency. This could not be predicted.

c. Reverse (Stage 0) Modifications

The two blocks of changes made to generate Stage 1 at positions 28-30 (NIK) and 94 (D) were mutated back to the NEWM sequences at positions 28-30 (TFS), 94 (R), or both positions 27-30 (TFS) and 94 (R). These constructs

were designated Stage 0-A, 0-B and 0-C, respectively. Each of these constructs was prepared by two-step PCR directed mutagenesis using the overlap/extension method of Ho et al., 1989 [74], as described for the Stage 2 Ser mutant, above. Stage 0-A and 0-B were generated from Stage 1; Stage 0-C was generated from Stage 0-A, as follows. For Stage 0-A, the change was from Asp to Arg at position 94. This change was accomplished using end primers, oligos 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] in conjunction with mutagenizing primers 915 [SEQ ID NO: 56] and 917 [SEQ ID NO: 57]. For stage 0-B, the changes were from Asn-Ile-Lys to Thr-Phe-Ser at positions 28-30. These changes were accomplished by using end primers 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] in conjunction with mutagenizing primers 918 [SEQ ID NO: 58] and 919 [SEQ ID NO: 59]. Finally, for stage 0-C, to the change of Asp to Arg at position 94 in Stage 0-A were added the changes were from Asn-Ile-Lys to Thr-Phe-Ser at positions 28-30. These changes were accomplished with the same end primers and mutagenizing primers described above for the Stage 0-B construct.

TABLE I

HEAVY CHAIN SEQUENCES

FR1

NEWM		?VQLXXSGPGLVRPSQTLSLTCTVSGSTFS
Humanized Anti-VLA4:		
STAGE O-A		QVQLQE.....FNIK
STAGE O-B		QVQLQE.....F...
STAGE O-C		QVQLQE.....F...
STAGE 1		QVQLQE.....FNIK
STAGE 2		QVQLQE.....FNIK
STAGE 3	(STAW)	QVQLQE.....FNIK
	(KAITAS)	QVQLQE.....FNIK
	(SSE)	QVQLQE.....FNIK
	(KRS)	QVQLQE.....FNIK
	(AS)	QVQLQE.....A..FNIK

FR2

NEWM		WVRQPPGRGLEWIG
Humanized Anti-VLA4:		
STAGE O-A		.....
STAGE O-B		.....
STAGE O-C		.....
STAGE 1		.....
STAGE 2		.....
STAGE 3	(STAW)	.....
	(KAITAS)	.....
	(SSE)	.....
	(KRS)	..K.R.....
	(AS)	.....

TABLE I (Cont'd)

FR3

NEWM			RVTMLVDTSKNQFSLRLSSVTAADTAVYYCAR
Humanized Anti-VLA4:			
STAGE O-A			.....
STAGE O-B			.....D
STAGE O-C			.....
STAGE 1			.....D
STAGE 2			.....S.....D
STAGE 3	(STAW)		.....S.TAW.....D
	(KAITAS)		KA.ITA...S.....D
	(SSE)		.....S.....SE.....D
	(KRS)		.....S.....D
	(AS)		.....S.....D

FR4

NEWM		WGQGSLVTVSS
Humanized Anti-VLA4:		
STAGE O-A		....TT.....
STAGE O-B		....TT.....
STAGE O-C		....TT.....
STAGE 1		....TT.....
STAGE 2		....TT.....
STAGE 3	(STAW)	....TT.....
	(KAITAS)	....TT.....
	(SSE)	....TT.....
	(KRS)	....TT.....
	(AS)	....TT.....

Note: X denotes Glx., ? denotes Q or E.

2. Light Chain Modifications

In our experience, the humanized light chain generally requires few, if any, modifications. However, in the preparation of humanized anti-VLA4 antibodies, it became apparent that the light chain of HP1/2 did require several empirical changes. For example, humanized heavy chain of the Stage 2 construct (the Ser mutant) with murine light chain was about 2.5 fold lower potency than murine HP1/2, while the same humanized heavy chain with humanized light chain was about 4-fold lower potency. The Stage 1 humanized V_K construct was designated VK1 (DQL) and the DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 30 and SEQ ID NO: 31, respectively. The DQL mutations arose from the PCR primer used in the initial cloning of the V_K region (see Example 1). Alterations were made in the light chain, generating two mutants, SVMDY and DQMDY (VK2 and VK3, respectively). The SVMDY mutant was prepared from the DQL sequence using oligos 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] for DY sequences with oligos 697 [SEQ ID NO: 60 and 698 [SEQ ID NO: 61] for SVM sequences. The VK2 (SVMDY) DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 62 and SEQ ID NO: 63, respectively. The DQMDY sequences were restored to the original REI framework sequences by two-step PCR-directed mutagenesis using end primers 10 [SEQ ID NO: 24] and 11 [SEQ ID NO: 26] with mutagenic primers 803 [SEQ ID NO: 64] and 804 [SEQ ID NO: 65], and using the SVMDY sequence as template. The VK3 (DQMDY) DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 66 and SEQ ID NO: 67, respectively. The change in the amino terminus (SVM versus DQM) is not relevant, and relates to the amino terminus of the murine light chain. The other two changes, D and Y, were made to improve potency, and did indeed do so as described in Example 7. An alignment of the CDR-grafted DQL (VK1), SVMDY (VK2)

and DQMDY (VK3) framework sequences as compared with the REI sequence is shown in Table II.

When the AS mutant heavy chain was combined with the improved light chain (SVMDY), the resulting humanized antibody was equipotent with murine HP1/2 as shown in Table III.

3. Alternative Humanized V_H and V_K Regions

Alternatively, a humanized V_H region sequence based on HP1/2 V_H region [SEQ ID NO: 5] may be prepared. One such alternative is designated V_H-PDLN. The DNA sequence of PDLN V_H and its translated amino acid sequence are set forth as SEQ ID NO: 68 and SEQ ID NO: 69, respectively.

In addition, an alternative humanized V_K region sequence based on the HP1/2 V_K region [SEQ ID NO: 9] may be prepared. One such alternative V_K sequence is designated V_K-PDLN and its translated amino acid sequence are set forth as SEQ ID NO: 70 and SEQ ID NO: 71, respectively.

The humanized V_H-PDLN was prepared by ligating 12 oligonucleotides, which together span the entire humanized variable region, and by screening for constructs having the correct sequence. The protocol is described in more detail below.

Oligonucleotides 370-119 through 370-130 (SEQ ID NO:72 through SEQ ID NO:83, respectively) (20 pmoles each) were dried down, and separately resuspended in 20 µl 1x Kinase Buffer containing 1 mM ATP and 1 µl T4 polynucleotide kinase (10 U/µl). The kinase reaction mixture was incubated for 1 hour at 37°C. The reaction was terminated by incubating at 70°C for 5 minutes.

The kinase-treated oligonucleotides were combined with each other (240 µl total) and ligated together with 26 µl 10 mM ATP and 2 µl T4 DNA ligase (10 U/µl), and the reaction mixture was incubated at room temperature for 6 hours. The ligation reaction mixture was extracted

with phenol:chloroform (1:1) saturated with TE buffer, and then ethanol precipitated and washed 5 times with 70% ethanol.

The dried and washed ethanol precipitate was resuspended in 50 μl 1x 150 mM Restriction Enzyme Buffer (10x 150 mM Restriction Enzyme Buffer is 100 mM Tris-HCl, pH 8.0, 1.5 M NaCl, 100 mM $MgCl_2$, 1 mg/ml gelatin, 10 mM dithiothreitol) and incubated with restriction enzymes BstE2 and PstI for 16 hours at 37°C. The digestion products were electrophoresed through a 2% agarose gel, and the band corresponding to 330 bp was excised. The fragment was eluted using GENECLEAN II® and the eluate was ethanol precipitated. The ethanol precipitate was resuspended in 20 μl TE buffer.

Next, the 330 bp fragment was ligated into vector pLCB7 which was prepared for ligation by digesting with PstI and BstE2, dephosphorylating the 5′ ends with calf alkaline phosphatase, fractionating on a low melting temperature agarose (LMA) gel, and excising the pLCB7/PstI/BstE2 LMA fragment. The pLCB7 LMA fragment was then ligated to the 330 bp oligonucleotide fragment encoding the humanized V_H region using T4 DNA ligase.

The ligation mixture was used to transform E. coli JA221(Iq) to ampicillin resistance. Colonies were grown up and mini-prep DNA was prepared. The recombinant plasmids were screened for the presence of an approximately 413 bp NotI/BstE2 fragment. DNA sequence analysis identified vector pMDR1023 as having the designed humanized V_H-PDLN sequence.

The humanized V_K-PDLN was prepared by ligating 12 oligonucleotides, which together span the entire humanized V_K-PDLN variable region, and by screening for constructs having the correct sequence. The protocol is described in more detail below.

Oligonucleotides 370-131 through 370-142 (SEQ ID NO:84 through SEQ ID NO:95, respectively)

(20 pmoles each) were dried down, and separately resuspended in 20 μl 1x Kinase Buffer containing 1 mM ATP and 1 μl T4 polynucleotide kinase (10 U/μl). The kinase reaction mixture was incubated for 1 hour at 37°C. The reaction was terminated by incubating at 70°C for 5 minutes.

The kinase-treated oligonucleotides were combined with each other (240 μl total) and ligated together with 26 μl 10 mM ATP and 2 μl T4 DNA ligase (10 U/μl), and the reaction mixture was incubated at room temperature for 6 hours. The ligation reaction mixture was extracted with phenol:chloroform (1:1) saturated with TE buffer, and then ethanol precipitated and washed 5 times with 70% ethanol.

The dried and washed ethanol precipitate was resuspended in 40 μl TE, then electrophoresed through a 2% agarose gel, and the band corresponding to 380 bp was excised. The fragment was eluted using GENECLEAN II® and the eluate was ethanol precipitated. The ethanol precipitate was resuspended in 20 μl TE buffer.

Next, the 380 bp fragment was ligated into vector pNN03, which was prepared for ligation by linearizing with HindIII and BamHI, dephosphorylating the 5′ ends with calf alkaline phosphatase, fractionating on a low melting temperature agarose gel, and excising the band corresponding to linearized pNN03 (2.7 kb). The linearized, dephosphorylated pNN03 was then ligated to the 380 bp oligonucleotide fragment encoding the humanized V_K region using T4 DNA ligase.

The ligation mixture was used to transform E. coli JA221(Iq) to ampicillin resistance. Colonies were grown up and mini-prep DNA was prepared. The recombinant plasmids were screened for the presence of the variable

region fragment. DNA sequence analysis identified vector pMDR1025 as having the designed humanized V_K-PDLN sequence.

When an antibody with a V_H-PDLN containing heavy chain and with a V_K-PDLN containing light chain was assayed for potency according to Example 7, the resulting humanized antibody was approximately equipotent with the murine HP1/2 antibody.

TABLE II

LIGHT CHAIN SEQUENCES

		FR1
	REI	DIQMTQSPSSLSASVGDRVTITC
	Humanized Anti-VLA4:	
	Construct VK1 (DQL)	...L.....
	Construct VK2 (SVMDY)	S.VM.....
	Construct VK3 (DQMDY)	D.QM.....
		FR2
	REI	WYQQTPGKAPKLLIY
	Humanized Anti-VLA4:	
	VK1 (DQL)	....K.....
	VK2 (SVMDY)	....K.....
	VK3 (DQMDY)	....K.....
		FR3
	REI	GVPSRFSGSGSGTDYTFTISSLQPEDIATYYC
	Humanized Anti-VLA4:	
	VK1 (DQL)	.....F.....
	VK2 (SVMDY)	...D.....Y...F.....
	VK3 (DQMDY)	...D.....Y...F.....
		FR4
	REI	FGQGTKLQIT
	Humanized Anti-VLA4:	
	VK1 (DQL)	.....VE.K
	VK2 (SVMDY)	.....VE.K
	VK3 (DQMDY)	.....VE.K

Example 5
Expression of Recombinant Anti-VLA4 Antibodies

Each of the V_H region sequences and V_K region sequences prepared according to Examples 1-4, are transferred into expression vectors with constant region sequences, and the vectors are transfected, preferably via electroporation, into mammalian cells. The heavy and light chain sequences may be encoded on separate vectors and co-transfected into the cells or alternatively heavy and light chain sequences may be encoded by and transfected as a single vector. Such a single vector will contain 3 expression cassettes: one for Ig heavy chain, one for Ig light chain and one for a selection marker. Expression levels of antibody are measured following transfection, as described below, or as described in Example 7.

V_H and V_K region sequences as described in Example 4, were inserted into various cloning and expression vectors. For the anti-VLA4 V_H region sequences, plasmids containing such sequences [as described in Examples 1-4] were digested with PstI and BstE2. The plasmid DNA after digestion with PstI and BstE2, was dephosphorylated and electrophoresed through 2% agarose gel. The band for ligation was excised and the DNA elected using the GENECLEAN™ technique (Bio101 Inc., LaJolla, California), ethanol precipitated and resuspended in 20 µl TE buffer (10mM Tris-HCl, 1mM Na_2 EDTA). Then, 10 µl of the resuspended DNA was used for ligation with the PstI/BstE2 digested V_H region sequence.

The ligation mixture was used to transform E. coli K 12 JA221 (Iq) to ampicillin resistance. E. coli K12 JA221 (Iq) cells have been deposited with the ATCC (accession number 68845). Recombinant colonies were screened for the presence of the V_H insert. Some of the plasmids containing such fragments were sequenced. The

V_H-containing plasmids were designated pBAG 184 (V_H-STAW), pBAG 183 (V_H-KAITAS), pBAG 185 (V_H-KRS), pBAG 207 (V_H-SSE) and pBAG 195 (V_H-AS), and were deposited in E. coli K12 J221 (Iq) cells with the ATCC as accession nos. 69110, 69109, 69111, 69116 and 69113, respectively. The plasmid containing alternative V_H-PDLN region was designated pMDR1023.

For the V_K region sequences, the DNA encoding these sequences were amplified for cloning and transformation using PCR. Prior to amplification, 20 pmoles of each of the V_K chain primers were kinased by incubation with T4 polynucleotide kinase at 37°C for 60 minutes by a conventional protocol. The kinase reactions were stopped by heating at 70°C for 10 minutes.

The PCR reactions each contained 10 µl 10X PCR buffer (10X PCR buffer is 100 mM Tris/HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin, 20 pmoles each of the appropriate kinased primers, 20 µl cDNA, 0.5 µl Taq polymerase (5 U/µl, Perkin Elmer-Cetus) and 49.5 µl H_2O. The PCR conditions were 30 cycles of incubation for: 1 minute at 94°C; 2 minutes at 40°C (for heavy chain PCR) or at 55°C (for light chain PCR); and 2 minutes at 72°C. For VK1-DQL, primers were 370-247 [SEQ ID NO: 96] and 370-210 [SEQ ID NO: 97]. For VK2-SVMDY, primers were 370-269 [SEQ ID NO: 98] and 370-210 [SEQ ID NO: 97]. For VK3-DQMDY, primers were 370-268 [SEQ ID NO: 99] and 370-210 [SEQ ID NO: 97].

The reaction mixtures were electrophoresed through 2% agarose gel, and the bands corresponding to the expected sizes of the light chain variable region (~330 bp) were excised with AgeI and BamHI. The DNA in those bands were eluted using the GENECLEAN™ technique (Bio101 Inc., LaJolla, California), ethanol precipitated and subsequently each resuspended in 20 µl TE buffer (10 mM Tris-HCl, 1 mM Na_2EDTA).

Klenow fragment of DNA polymerase (New England Biolabs, 5 U/μl) (1 μl) was added to the purified PCR fragments in a reaction volume of 25 μl containing 1x ligation buffer (10x ligation buffer is 0.5 M Tris/HCl, pH 7.5, 100 mM $MgCl_2$ and 40 mM DTT) and 0.125 mM each of dXTPs and the reaction incubated at room temperature for 15 minutes. The reaction was terminated by incubation at 70°C for 5 minutes, and then stored on ice.

The fragment from each PCR reaction is ligated to a plasmid such as pNN03 or a plasmid derived from pNN03 such as pLCB7, which had been previously linearized by EcoRV, dephosphorylated and fractionated through low temperature melting agarose. Such plasmids, including pNN03 and pLCB7 have been described in co-pending and co-assigned (Burkly et al., U.S. Ser. No. 07/916,098, filed July 24, 1992 [75]).

The ligation mixture was used to transform E.coli K12 JA221(Iq) to ampicillin resistance. E.coli K12 JA221(Iq) cells are deposited with American Type Culture Collection (accession number 68845). Recombinant colonies were screened for the presence of the V_K insert. Some of the plasmids containing such fragments were sequenced. The V_K-containing plasmids were designated pBAG 190 (VK1-DQL), pBAG 198 (VK2-SVMDY) and pBAG 197 (VK3-DQMDY), and were deposited in E. coli K12 JA 221 (Iq) cells with the ATCC as accession nos. 69112, 69115 and 69114, respectively. The plasmid containing the alternative V_K (PDLN) region was designated pMDR 1025.

In a series of experiments, the expression vectors encoding recombinant anti-VLA4 heavy and light chains are transfected via electroporation and the cells are then cultured for 48 hours. After 48 hours of culture, the cells are radiolabelled using ^{35}S-cysteine overnight and then the cell extracts and conditioned media are immunoprecipitated by incubation with protein A-Sepharose. The protein A-Sepharose is washed and the

bound proteins are eluted with SDS-PAGE loading buffer. The samples are analyzed via electrophoresis through 10% SDS-PAGE gels under reducing conditions. In this way, light chain expression is detected only as a consequence of the light chains being associated with the heavy chains. The expected sizes of the heavy and light chains as visualized in the 10% gels are 50 kD and 25 kD, respectively.

Since recombinant anti-VLA4 antibody molecules, prepared as described in Examples 1-4, may be stably expressed in a variety of mammalian cell lines, it is possible to express recombinant antibody genes in nonsecreting myeloma or hybridoma cell lines under the control of Ig-gene promoters and enhancers or in non-lymphoid cells, such as Chinese hamster ovary (CHO) cells, in conjunction with vector amplification using DHFR selection. Recently, Bebbington et al., 1992 [76] have described a method for the high-level expression of a recombinant antibody from myeloma cells using a glutamine synthetase gene as an amplifiable marker. This GS expression system is most preferred for the production of recombinant anti-VLA4 antibody molecules according to the present invention. The methods, vectors with hCMV promoters and with 5' untranslated sequences from the hCMV-MIE genes including cell lines (most preferably NSO) and media for GS expression of recombinant antibodies is described in detail in Bebbington et al., 1992 [76], WO86/05807 [77], WO87/04462 [78], WO89/01036 [79] and WO89/10404 [80].

In accordance with the teachings of these publications, NSO cells were transfected with a heavy chain sequence having the VH-AS region sequence [SEQ ID NO: 54] and a light chain sequence having the VK-SVMDY sequence [SEQ ID NO: 66] to obtain a stable cell line secreting a humanized recombinant anti-VLA4 antibody with high potency comparable to the murine HP1/2 antibody.

This cell line has been deposited with the ATCC on November 3, 1992 and given accession no. CRL 11175. The AS/SVMDY humanized antibody is at least equipotent with or perhaps more potent than the murine HP1/2 antibody.

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Example 6

Purification of MAbs from Conditioned Media for Assay

To obtain accurate values for half-maximal binding or inhibition, stock solutions of purified antibodies are needed at known concentrations. Stable cell lines secreting the antibodies of interest were made and the humanized recombinant anti-VLA4 antibodies were purified from conditioned medium using conventional protein A chromatography. The concentration of the purified antibodies is assessed by their absorption coefficient at 280 nm, which is well established for antibodies.

A cell line producing a humanized anti-VLA4 antibody is grown in roller bottles in Dulbecco's modified Eagle medium containing 10% fetal bovine serum. A 2 liter batch of conditioned medium is used for each purification run. Cells are removed from the medium by centrifugation in a RC-3B preparative centrifuge (4K, 30 minutes, H4000 rotor) and the supernatant is filtered first through a 0.45 μ membrane and then through a 0.22 μ membrane. The medium is stored at 4°C until it can be processed.

Two liters of conditioned medium is concentrated to 220 ml in a spiral ultrafiltration unit (Amicon, Corp., Cherry Hill Drive, Danvers, MA 01923) that is equipped with an S1Y30 (YM30) Diaflo cartridge. The concentrate is diluted with 400 ml of protein A binding buffer (3M NaCl, 1.5M glycine pH 8.9) and again concentrated to 200 ml. The concentrate is treated in batch with 0.5 ml Fast Flow Protein A Sepharose 4 (Pharmacia, Inc., 800 Centennial Avenue, Piscataway, NJ 08854) using a raised stir bar to agitate the mixture. After an overnight incubation at 4°C, the resin is collected by centrifugation (5 minutes, 50 g), washed twice with 20 volumes of protein A binding buffer (using centrifugation to recover the resin), and transferred to a column for subsequent treatment. The column is washed four times

with 0.5 ml of protein A binding buffer, two times with 0.25 ml of PBS, and the IgG is eluted with Pierce IgG elution buffer (Pierce Chemical Co., Rockford, IL. 61105 Cat No. 21004Y or 21009Y). 180 µl fractions are collected, which are neutralized with 20 µl of 1M HEPES pH 7.5. Fractions are analyzed for absorbance at 280 nm and by SDS-PAGE. The gel is stained with Coomassie blue. Peak fractions are pooled. 100 µl (14 ml/ml) is diluted with 100 µl of PBS and subjected to gel filtration on a Superose 6 FPLC column (Pharmacia, Inc., 800 Centennial Avenue, Piscataway, NJ 08854) in PBS. The column is run at 20 ml/hour and 1.5 minute fractions are collected. Peak column fractions are pooled, aliquoted, frozen on dry ice, and stored at -70°C. SDS-polyacrylamide gel profile of the final product is obtained under reducing and non-reducing conditions. In some cases when the sample is analyzed under non-reducing conditions, about 10% of the product is not an intact antibody. Studies in these cases indicate that this product is a heavy-light chain dimer. This has been previously recognized as a problem with IgG4 antibodies.

Example 7

Determination of Relative Binding Affinities of Humanized Recombinant Anti-VLA4 Antibodies

Recombinant antibodies according to the present invention are purified, as described in Example 6, and are assayed to determine their specificity for VLA4 and their binding affinity or potency. In particular, the potency of a recombinant anti-VLA4 antibody was assessed by calculating the half-maximal binding constant (reported as ng/ml or μg/ml of purified antibody) using two different assays described as follows.

1. Inhibition of VLA4-dependent adhesion to VCAM1

The critical function of an anti-VLA4 antibody is defined by the ability to inhibit the VCAM1/VLA4 adhesion pathway. It has been previously shown (Lobb et al., 1991a, [81]) that purified recombinant soluble VCAM1 (rsVCAM1) can be immobilized on plastic and is a functional adhesion molecule. Immobilized rsVCAM1 binds VLA4-expressing cells such as the human B cell line Ramos, and this binding can be inhibited by MAbs to VCAM1, such as 4B9 or MAbs to VLA4, such as HP1/2. This assay provides a reproducible method to assess the potency of any humanized recombinant antibody. Briefly, the antibody solution is diluted, and the serial antibody dilutions are incubated with Ramos cells, which are then incubated with rsVCAM1-coated plates. The Ramos cells are fluorescently labelled as described by Lobb, 1991b [82], and binding assessed by fluorescence in 96 well cluster plates according to the following protocol.

Recombinant soluble VCAM1 was prepared and purified essentially as described by Lobb et al., 1991a [81]. Soluble VCAM is diluted to 10 μg/ml in 0.05 M $NaHCO_3$, (15mM $NaHCO_3$, 35mM Na_2CO_3) pH 9.2. Then 50 μl/well is added into a Linbro Titertek polystyrene 96 well plate, flat bottom, Flow Labs catalog #76-231-05. The plate is incubated at 4°C overnight.

Following this incubation, the contents of the wells are removed by inverting and blotting the plate. To the empty wells, 100 µl/well of 1% of BSA in PBS, 0.02% NaN_3 is added for 1 hour or longer at room temperature. If the plate is not to be used immediately, it can be blocked and stored for one week at 4°C. BSA is added to some wells to assess non-specific binding.

For binding quantitation, VLA4 presenting cells, preferably Ramos cells, should be prelabelled. The cells may be radiolabelled or fluorescently labelled. For radiolabelling, prelabelling of the cells may be done overnight using ^{3}H-thymidine (0.5 µCi/ml). Alternatively, and preferably, the cells are preincubated with BCECF-AM (chemical name: 2',7'-bis-(2-carboxyethyl)-5(and -6) carboxyfluorescein, acetoxymethyl ester, Molecular Probes Inc., Eugene, Oregon, catalog #B-1150). For this method, cells are suspended to 5 x 10^6/ml, 2 µM BCECF-AM is added and the mixture is incubated for 30 minutes at 37°C. Following either method, the cells are washed with RPMI, 2% FBS, pH 7.4. RPMI with 1% FBS may also be used.

For the binding study, 2-4 x 10^6 cells/ml in RPMI, 2% FBS are resuspended, then 50 µl of labelled cells are added per well for 10 minutes of binding at room temperature.

After the 10 minute incubation, the contents of the wells are removed by inversion and the plates washed 1-2 times gently with RPMI, 2% FBS. When examined under a light microscope, BSA blank wells should have very few cells bound. A brief inverted spin may be included to remove cells not firmly attached and the plates may be washed again 1-2 times.

For the BCECF-AM method, 100 µl of 1% NP40 is added to each well to solubilize the cells and then the plate is read on a fluorescence plate scanner. (If the radiolabelling method is used, 100 µl of 0.1% NaOH is

added to each well and then the contents of each well are transferred to scintillation vials containing cocktail).

A volume of 50 μl of labelled cells should be counted to obtain a total known value added to each well. Then the 50 μl of labelled cells are added to either a well containing only 100 μl of 1% NP40 or to a scintillation vial depending on the method used.

For antibody blocking studies, 100 μl/well of murine HP1/2 MAb (anti-VLA4) typically at 10 μg/ml in RPMI, 2% FBS are added to the rsVCAM1 coated plates and incubated for 30 minutes at room temperature prior to cell binding as described above. MAb HP1/2 (anti-VLA4) or any recombinant humanized anti-VLA4 antibody prepared as described herein must be preincubated with labelled cells for 30 minutes at room temperature prior to the cell binding. Concentrations of the antibodies preincubated will vary, but generally concentrations were in the range of about 1 μg/ml.

In these adhesion assays, murine HP1/2 inhibits Ramos cell binding completely at about 40 ng/ml, and half maximally at about 15 ng/ml (10 μM). The results of adhesion assays as represented by the calculated half-maximal binding constants using humanized recombinant anti-VLA4 antibodies made according to the present invention are shown in Table III. The number (n) of experiments performed for each value is indicated for the recombinant humanized antibodies. As discussed below, these results generally compare well with the results obtained with the FACS binding assay.

The potency of recombinant Stage 0, Stage 1, Stage 2 and Stage 3 antibodies having the VK1 (DQL) light chain that had been purified from stably transfected YB2/0 cell lines was measured in the adhesion assay, as shown in Table III. The results showed that there was no inhibition detected in concentrations up to 1 μg/ml (1000 ng/ml) with the Stage 0-B and 0-C humanized antibodies.

The results with the recombinant Stage 3 antibodies STAW and AS having the improved VK2 (SVMDY) light chain showed that the AS/SVMDY antibody was at least equipotent and perhaps more potent than the murine HP1/2 antibody. Certain Stage 2 and Stage 3 constructs showed potencies of about 20% to about 100% of the potency of the murine HP1/2 antibody.

2. FACS Assays

The binding of humanized recombinant antibodies to the cell surface can be assessed directly by fluorescence activated cell sorter (FACS) analysis, using fluorescently labelled antibodies. This is a standard technique that also provides half-maximal binding information following dose response measurements. The FACS methods are described in Lobb et al., 1991b [82].

Briefly, 25 μl cells (4 x 10^6/ml in FACS buffer (PBS 2% FBS, 0.1% NaN_3) on ice are added to 5 μl of 5 μg/ml FITC or phycoerythrin (PE) conjugated antibody in FACS buffer, and incubated in V-bottomed microtiter wells on ice for 30 minutes. To the wells, 125 μl of FACS buffer is added, the plates are centrifuged at 350 x g for 5 minutes, and the supernatant is shaken off. To each well is added 125 μl FACS buffer, then the cells are transferred to 12 x 75 mm Falcon polystyrene tubes and resuspended to a final volume of 250 μl in FACS buffer. The mixture is analyzed on a Becton Dickinson FACStar. The results of the FACS assays as represented by the calculated half-maximal binding constructs using humanized recombinant anti-VLA4 antibodies made according to the present invention are shown in Table III and the number (n) of experiments performed for each value is indicated for the humanized antibodies. Table III also shows the potency calculated from the combined adhesion and FACS assays. Murine HP1/2 binds half-maximally to Ramos cells at 15 ng/ml. The AS/SVMDY humanized antibody binds half-maximally to Ramos cells at 12 ng/ml. Thus,

the two assays (i.e., adhesion and FACS assays) show an excellent correlation for the murine antibody and the humanized AS/SVMDY antibody.

TABLE III

SUMMARY OF HALF-MAXIMAL BINDING CONSTANTS FOR HUMANIZED RECOMBINANT ANTI-VLA4 ANTIBODIES

Antibody	Adhesion Assay	FACS Assay	Combination
Murine HP1/2	15 ng/ml	15 ng/ml	15 ng/ml
Stage 0 (Humanized heavy chain)	>1000 ng/ml (n=3)	-	-
Stage 1 (Humanized heavy chain)	228 ng/ml (n=6)	-	228 ng/ml (n=6)
Stage 2 (Ser mutant)	56 ng/ml (n=14)	47 ng/ml (n=6)	60 ng/ml (n=20)
Stage 3			
(STAW)	30 ng/ml (n=3)	33 ng/ml (n=3)	32 ng/ml (n=6)
(KAITAS)	85 ng/ml (n=2)	100 ng/ml (n=1)	90 ng/ml (n=3)
(SSE)	100 ng/ml (n=2)	40 ng/ml (n=1)	80 ng/ml (n=3)
(KRS)	50 ng/ml (n=2)	70 ng/ml (n=1)	57 ng/ml (n=3)
(AS)	28 ng/ml (n=2)	14 ng/ml (n=2)	21 ng/ml (n=4)
Constructs with improved light chain			
STAW/SVMDY	25 ng/ml (n=4)	35 ng/ml (n=3)	29 ng/ml (n=7)
AS/SVMDY	12 ng/ml (n=2)	12 ng/ml (n=2)	12 ng/ml (n=4)

Deposits

The following plasmids in E. coli K12 J221 (Iq) cells were deposited under the Budapest Treaty with American Type Culture Collection (ATCC), Rockville, Maryland (USA) on October 30, 1992. The deposits are identified as follows:

Plasmid	Accession No.
pBAG 184 (V_H-STAW)	69110
pBAG 183 (V_H-KAITAS)	69109
pBAG 185 (V_H-KRS)	69111
pBAG 207 (V_H-SSE)	69116
pBAG 195 (V_H-AS)	69113
pBAG 190 (VK1-DQL)	69112
pBAG 198 (VK2-SVMDY)	69115
pBAG 197 (VK3-DQMDY)	69114

In addition, an NSO cell line producing humanized recombinant anti-VLA4 antibody was deposited under the Budapest Treaty with American Type Culture Collection (ATCC), Rockville, Maryland (USA) on November 3, 1992. The deposit was given ATCC accession no. CRL 11175.

Sequences

The following is a summary of the sequences set forth in the Sequence Listing:

SEQ ID NO:1 DNA sequence of CG1FOR primer

SEQ ID NO:2 DNA sequence of CK2FOR primer

SEQ ID NO:3 DNA sequence of VH1BACK primer

SEQ ID NO:4 DNA sequence of VH5BACK primer

SEQ ID NO:5 DNA sequence of HP1/2 heavy chain variable region

SEQ ID NO:6 Amino acid sequence of HP1/2 heavy chain variable region

SEQ ID NO:7 DNA sequence of VK1BACK primer

SEQ ID NO:8 DNA sequence of VK7BACK primer

SEQ ID NO:9 DNA sequence of HP1/2 light chain variable region

SEQ ID NO:10 Amino acid sequence of HP1/2 light chain variable region

SEQ ID NO:11 DNA sequence of VH1FOR primer

SEQ ID NO:12 DNA sequence of VK3BACK primer

SEQ ID NO:13 DNA sequence of VK1FOR primer

SEQ ID NO:14 DNA sequence of VH insert in M13VHPCR1

SEQ ID NO:15 Amino acid sequence of VH insert in M13VHPCR1

SEQ ID NO:16 DNA sequence of VK insert in M13VKPCR2

SEQ ID NO:17 Amino acid sequence of VK insert in M13VKPCR2

SEQ ID NO:18 DNA sequence of OLIGO598

SEQ ID NO:19 DNA sequence of OLIGO599

SEQ ID NO:20 DNA sequence of OLIGO600

SEQ ID NO:21 DNA sequence of OLIGO605

SEQ ID NO:22 DNA sequence of OLIGO606

SEQ ID NO:23 DNA sequence of OLIGO607

SEQ ID NO:24 DNA sequence of OLIGO10

SEQ ID NO:25 DNA sequence of OLIGO385

SEQ ID NO:26 DNA sequence of OLIGO11

SEQ ID NO:27 DNA sequence of OLIGO391

SEQ ID NO:28 DNA sequence of Stage 1 heavy chain variable region

SEQ ID NO:29 Amino acid sequence of Stage 1 heavy chain variable region

SEQ ID NO:30 DNA sequence of VK1 (DQL) light chain variable region

SEQ ID NO:31	Amino acid sequence of VK1 (DQL) light chain variable region
SEQ ID NO:32	DNA sequence of Stage 2 heavy chain variable region
SEQ ID NO:33	Amino acid sequence of Stage 2 heavy chain variable region
SEQ ID NO:34	DNA sequence of OLIGO684
SEQ ID NO:35	DNA sequence of OLIGO683
SEQ ID NO:36	DNA sequence of OLIGO713
SEQ ID NO:37	DNA sequence of OLIGO716
SEQ ID NO:38	DNA sequence of STAW heavy chain variable region
SEQ ID NO:39	Amino acid sequence of STAW heavy chain variable region
SEQ ID NO:40	DNA sequence of OLIGO706
SEQ ID NO:41	DNA sequence of OLIGO707
SEQ ID NO:42	DNA sequence of KAITAS heavy chain variable region
SEQ ID NO:43	Amino acid sequence of KAITAS heavy chain variable region
SEQ ID NO:44	DNA sequence of OLIGO768
SEQ ID NO:45	DNA sequence of OLIGO769
SEQ ID NO:46	DNA sequence of SSE heavy chain variable region
SEQ ID NO:47	Amino acid sequence of SSE heavy chain variable region
SEQ ID NO:48	DNA sequence of OLIGO704
SEQ ID NO:49	DNA sequence of OLIGO705
SEQ ID NO:50	DNA sequence of KRS heavy chain variable region
SEQ ID NO:51	Amino acid sequence of KRS heavy chain variable region
SEQ ID NO:52	DNA sequence of OLIGO745

SEQ ID NO:53	DNA sequence of OLIGO746
SEQ ID NO:54	DNA sequence of AS heavy chain variable region
SEQ ID NO:55	Amino acid sequence of AS heavy chain variable region
SEQ ID NO:56	DNA sequence of OLIGO915
SEQ ID NO:57	DNA sequence of OLIGO917
SEQ ID NO:58	DNA sequence of OLIGO918
SEQ ID NO:59	DNA sequence of OLIOG919
SEQ ID NO:60	DNA sequence of OLIGO697
SEQ ID NO:61	DNA sequence of OLIGO698
SEQ ID NO:62	DNA sequence of VK2 (SVMDY) light chain variable region
SEQ ID NO:63	Amino acid sequence of VK2 (SVMDY) light chain variable region
SEQ ID NO:64	DNA sequence of OLIGO803
SEQ ID NO:65	DNA sequence of OLIGO804
SEQ ID NO:66	DNA sequence of VK3 (DQMDY) light chain variable region
SEQ ID NO:67	Amino acid sequence of VK3 (DQMDY) light chain variable region
SEQ ID NO:68	DNA sequence of PDLN heavy chain variable region
SEQ ID NO:69	Amino acid sequence of PDLN heavy chain variable region
SEQ ID NO:70	DNA sequence of PDLN light chain variable region
SEQ ID NO:71	Amino acid sequence of PDLN light chain variable region
SEQ ID NO:72	DNA sequence of Oligo 370-119
SEQ ID NO:73	DNA sequence of Oligo 370-120
SEQ ID NO:74	DNA sequence of Oligo 370-121

SEQ ID NO:75 DNA sequence of Oligo 370-122

SEQ ID NO:76 DNA sequence of Oligo 370-123

SEQ ID NO:77 DNA sequence of Oligo 370-124

SEQ ID NO:78 DNA sequence of Oligo 370-125

SEQ ID NO:79 DNA sequence of Oligo 370-126

SEQ ID NO:80 DNA sequence of Oligo 370-127

SEQ ID NO:81 DNA sequence of Oligo 370-128

SEQ ID NO:82 DNA sequence of Oligo 370-129

SEQ ID NO:83 DNA sequence of Oligo 370-130

SEQ ID NO:84 DNA sequence of Oligo 370-131

SEQ ID NO:85 DNA sequence of Oligo 370-132

SEQ ID NO:86 DNA sequence of Oligo 370-133

SEQ ID NO:87 DNA sequence of Oligo 370-134

SEQ ID NO:88 DNA sequence of Oligo 370-135

SEQ ID NO:89 DNA sequence of Oligo 370-136

SEQ ID NO:90 DNA sequence of Oligo 370-137

SEQ ID NO:91 DNA sequence of Oligo 370-138

SEQ ID NO:92 DNA sequence of Oligo 370-139

SEQ ID NO:93 DNA sequence of Oligo 370-140

SEQ ID NO:94 DNA sequence of Oligo 370-141

SEQ ID NO:95 DNA sequence of Oligo 370-142

SEQ ID NO:96 DNA sequence of VK1-DQL primer 370-247

SEQ ID NO:97 DNA sequence of VK1-DQL primer 370-210

SEQ ID NO:98 DNA sequence of VK2-SVMDY primer 370-269

SEQ ID NO:99 DNA sequence of VK3-DQMDY primer 370-268

While we have hereinbefore described a number of embodiments of this invention, it is apparent that our basic embodiments can be altered to provide other

embodiments that utilize the compositions and processes of this invention. Therefore, it will be appreciated that the scope of this invention includes all alternative embodiments and variations which are defined in the foregoing specification and by the claims appended hereto; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

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Each of the above-listed references is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lobb, Roy R.; Carr, Frank J.; Tempest, Philip R.

(ii) TITLE OF INVENTION: Recombinant Anti-VLA4 Antibody Molecules

(iii) NUMBER OF SEQUENCES: 99

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(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: McNicholas, Janet M.
(B) REGISTRATION NUMBER: 32,918
(C) REFERENCE/DOCKET NUMBER: 92,445/D012 US

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312-715-1000
(B) TELEFAX: 312-715-1234

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
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(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "CG1FOR PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "CK2FOR PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

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(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VH1BACK PCR primer"

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(2) INFORMATION FOR SEQ ID NO:4:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VK5BACK PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG159 insert: HP1/2 heavy chain variable region; 1, 3 & 6 = E, Q & E in HP1/2"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
  2               6                  11                  16

GTC AAG TTG TCC TGC ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT      96
Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
             21                  26                  31

ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA     144
Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
         36                  41                  46

AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC CCG AAG TTC CAG     192
Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
     51                  56                  61

GTC AAG GCC ACT ATT ACA GCG GAC ACG TCC TCC AAC ACA GCC TGG CTG     240
Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
 66                  71                  76                  81
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CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCC GTC TAC TAC TGT GCA        288
Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                86                  91                  96

GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC TTC TGG GGC CAA        336
Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
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GGG ACC ACG GTC ACC GTC TCC TCA                                      360
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser
  2                 6                  11                  16

Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr
            21                  26                  31

Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly
        36                  41                  46

Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe Gln
    51                  56                  61

Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Trp Leu
66                  71                  76                  81

Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
                86                  91                  96

Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly Gln
            101                 106                 111

Gly Thr Thr Val Thr Val Ser Ser
        116                 121
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VK1BACK PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GACATTCAGC TGACCCAGTC TCCA 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VK7BACK PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTGAATTCGG AGTTGATGGG AACATTGTAA TG 32

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 318 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..318
(D) OTHER INFORMATION: /product= "HP1/2 light chain variable region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "pBAG172 insert: HP1/2 light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
AGT ATT GTG ATG ACC CAG ACT CCC AAA TTC CTG CTT GTT TCA GCA GGA        48
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1               5                  10                  15

GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG ACT AAT GAT        96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
            20                  25                  30

GTA GCT TGG TAC CAA CAG AAG CCA GGG CAG TCT CCT AAA CTG CTG ATA       144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

TAT TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC TTC ACT GGC       192
Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60

AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC ACT GTG CAG GCT       240
Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65                  70                  75                  80

GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT TAT AGC TCT CCG TAC       288
Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
                85                  90                  95

ACG TTC GGA GGG GGG ACC AAG CTG GAG ATC                              318
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45
```

Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VH1FOR PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VK3BACK PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GACATTCAGC TGACCCA 17

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "VK1FOR PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTTAGATCTC CAGCTTGGTC CC 22

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 823 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..621
(D) OTHER INFORMATION: /note= "VH insert in M13 VHPCR1"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 261..621

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: join(122..167, 250..260)

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 261..621

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(122..167, 250..621)

(ix) FEATURE:
(A) NAME/KEY: TATA_signal
(B) LOCATION: 38..45

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 351..365
(D) OTHER INFORMATION: /note= "CDR1"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 408..458
(D) OTHER INFORMATION: /note= "CDR2"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 555..587
(D) OTHER INFORMATION: /note= "CDR3"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: (621^622)
(D) OTHER INFORMATION: /note= "splice to constant region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
AAGCTTATGA ATATGCAAAT CCTCTGAATC TACATGGTAA ATATAGGTTT GTCTATACCA      60

CAAACAGAAA AACATGAGAT CACAGTTCTC TCTACAGTTA CTGAGCACAC AGGACCTCAC     120

C ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA  G      167
  Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  -19             -15                 -10                 -5

GTAAGGGGCT CACAGTAGCA GGCTTGAGGT CTGGACATAT ATATGGGTGA CAATGACATC     227

CACTTTGCCT TTCTCTCCAC AG  GT GTC CAC TCC CAG GTC CAA CTG CAG GAG       278
                         Gly Val His Ser Gln Val Gln Leu Gln Glu
                              -3          1               5

AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG ACC CTG AGC CTG ACC TGC       326
Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser Leu Thr Cys
            10                  15                  20

ACC GTG TCT GGC AGC ACC TTC AGC AGC TAC TGG ATG CAC TGG GTG AGA       374
Thr Val Ser Gly Ser Thr Phe Ser Ser Tyr Trp Met His Trp Val Arg
        25                  30                  35

CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT GGA AGG ATT GAT CCT AAT       422
Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Asn
    40                  45                  50

AGT GGT GGT ACT AAG TAC AAT GAG AAG TTC AAG AGC AGA GTG ACA ATG       470
Ser Gly Gly Thr Lys Tyr Asn Glu Lys Phe Lys Ser Arg Val Thr Met
55                  60                  65                  70
```

```
CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC CTG AGA CTC AGC AGC GTG        518
Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg Leu Ser Ser Val
                75                  80                  85

ACA GCC GCC GAC ACC GCG GTC TAT TAT TGT GCA AGA TAC GAT TAC TAC        566
Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Tyr Asp Tyr Tyr
            90                  95                  100

GGT AGT AGC TAC TTT GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC        614
Gly Ser Ser Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
        105                 110                 115

TCC TCA G                                                             621
Ser Ser
    120
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19                 -15                 -10                  -5

Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
                1                   5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Ser Thr Phe
     15                  20                  25

Ser Ser Tyr Trp Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Asn Ser Gly Gly Thr Lys Tyr Asn
                50                  55                  60

Glu Lys Phe Lys Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
            65                  70                  75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
        80                  85                  90

Tyr Tyr Cys Ala Arg Tyr Asp Tyr Tyr Gly Ser Ser Tyr Phe Asp Tyr
     95                  100                 105
```

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 594 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..632
(D) OTHER INFORMATION: /note= "VK insert in M13 VKPCR2"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 273..594

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: join(134..179, 262..272)

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 273..594

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(134..179, 262..594)

(ix) FEATURE:
(A) NAME/KEY: TATA_signal
(B) LOCATION: 50..57

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 342..374
(D) OTHER INFORMATION: /note= "CDR1"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 420..440
(D) OTHER INFORMATION: /note= "CDR2"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 537..563
(D) OTHER INFORMATION: /note= "CDR3"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: (594^595)
(D) OTHER INFORMATION: /note= "splice to constant region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
CTCTTAAACT TCAAGCTTAT GAATATGCAA ATCCTCTGAA TCTACATGGT AAATATAGGT      60

TTGTCTATAC CACAAACAGA AAAACATGAG ATCACAGTTC TCTCTACAGT TACTGAGCAC     120

ACAGGACCTC ACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA         169
               Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala
               -19             -15                 -10

ACA GCT ACA  G GTAAGGGGCT CACAGTAGCA GGCTTGAGGT CTGGACATAT            219
Thr Ala Thr
        -5

ATATGGGTGA CAATGACATC CACTTTGCCT TTCTCTCCAC AG  GT GTC CAC TCC         272
                                               Gly Val His Ser
                                                  -3

GAC ATC CAG CTG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT        320
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15

GAC AGA GTG ACC ATC ACC TGT AGA GCC AGC GGT AAC ATC CAC AAC TAC        368
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
            20                  25                  30

CTG GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CTG CTG ATC        416
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

TAC TAC ACC ACC ACC CTG GCT GAC GGT GTG CCA AGC AGA TTC AGC GGT        464
Tyr Tyr Thr Thr Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC CAG CCA        512
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

GAG GAC ATC GCC ACC TAC TAC TGC CAG CAC TTC TGG AGC ACC CCA AGG        560
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Arg
                85                  90                  95

ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA C                         594
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19             -15                 -10                  -5

Val His Ser Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala
                1                   5                   10

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile
     15                  20                  25

His Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30                  35                  40                  45

Leu Leu Ile Tyr Tyr Thr Thr Thr Leu Ala Asp Gly Val Pro Ser Arg
                50                  55                  60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
            65                  70                  75

Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His Phe Trp Ser
        80                  85                  90

Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
     95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 598 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGTCTCACCC AGTGCATATA GGTGTCTTTA ATGTTGAAGC CAGACACGCT GCAG 54

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 599 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAGCATTGTC ACTCTGACCT GGAACTTCGG GTCATATTTA GTATCGCCAC TCGCAGGATC 60

AATCCTTCCA A 71

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 600 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGTCCCTTGG CCCCAGAAGT CCAGAGCATA TCCCGTTGAT ACCCACATTC CGTCTGCACA 60

ATAATAGACC 70

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 605 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCCCTTGGCC GAACGTGTAC GGAGAGCTAT AATCCTGCTG GCAGTAGTAG G 51

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 606 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATCTGCTTGG GCACACCAGT GTAGCGATTG GATGCATAGT AGATCAGCAG CT 52

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 607 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCTGCTGGTA CCAAGCTACA TCATTAGTCA CACTCTGACT GGCCTTACAG GTGATGGTCA 60

C 61

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence OLIGO 10 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of OLIGO 385 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCGGGCCTCT TCGCTATTACGC 22

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of OLIGO 11 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AACAGCTATG ACCATG 16

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of OLIGO 391 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTCTCTCAGG GCCAGGCGGT GA 22

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pMDR1019 insert: Stage 1 heavy chain variable region "

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT        48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                 -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA        96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
               1               5                  10

CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT       144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT       192
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC       240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                 50                  55                  60

CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC       288
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
             65                  70                  75

CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC       336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
         80                  85                  90

TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC       384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC           429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19              -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
               1                 5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                 50                  55                  60

Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
             65                  70                  75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
         80                  85                  90

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 386 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..386

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..386

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG190 insert: VK1 (DQL) light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC GGT        48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19             -15                 -10                 -5

GTT CAC TCC GAC ATC CAG CTG ACC CAG AGC CCA AGC AGC CTG AGC GCC        96
Val His Ser Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala
            1               5                  10

AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT CAG AGT GTG       144
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val
     15                  20                  25

ACT AAT GAT GTA GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG       192
Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30                  35                  40                  45


CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT GGT GTG CCA AGC AGA       240
Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg
                50                  55                  60

TTC AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC       288
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
            65                  70                  75

CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC       336
Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser
         80                  85                  90

TCT CCG TAC ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGT AAG TG    386
Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys
     95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19              -15                 -10                  -5

Val His Ser Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala
                 1                   5                  10

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val
     15                  20                  25

Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30                  35                  40                  45

Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg
                 50                  55                  60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
             65                  70                  75

Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser
         80                  85                  90

Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys
     95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pMDR1028 insert: Stage 2 heavy chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT      48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19                 -15                 -10                  -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA      96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
                 1               5                  10

CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT     144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT     192
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC     240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                 50                  55                  60

CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AGC AAC     288
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
             65                  70                  75

CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC     336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
         80                  85                  90

TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC     384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC         429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19              -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
               1                 5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
      15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                   50                  55                  60

Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
               65                  70                  75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
          80                  85                  90

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
      95                  100                 105

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 684 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AGACACCAGC AGCAACCAGT TCAG 24

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 683 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGAACTGGTT GCTGCTGGTG TCTA 24

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 713 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

ACCAGCAGCA ACACAGCCTG GCTGAGACTC AGCAGCG 37

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 716 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCTGAGTCTC AGCCAGGCTG TGTTGCTGCT GGTGTCGA 38

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG184 insert: STAW heavy chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT        48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                 -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA        96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
              1               5                  10

CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT       144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT       192
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45
```

```
GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC      240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                50                  55                  60

CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AGC AAC      288
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
            65                  70                  75

ACA GCC TGG CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC      336
Thr Ala Trp Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
        80                  85                  90

TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC      384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
    95                  100                 105

TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC          429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19                 -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
                1                   5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
    15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                50                  55                  60

Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
            65                  70                  75

Thr Ala Trp Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
        80                  85                  90
```

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
95 100 105

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 706 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGTTCCAGGT CAAAGCGACA ATTACGGCAG ACACCAGCAA 40

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 707 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTTGCTGGTG TCTGCCGTAA TTGTCGCTTT GACCTGGAAC 40

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG183 insert: KAITAS heavy chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT          48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                 -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA          96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
            1               5                  10

CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT         144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT         192
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC         240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                50                  55                  60

CCG AAG TTC CAG GTC AAA GCG ACA ATT ACG GCA GAC ACC AGC AGC AAC         288
Pro Lys Phe Gln Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn
            65                  70                  75

CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC         336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
        80                  85                  90
```

```
TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC      384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
 95                  100                 105

TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC      429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
              1                5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                 50                  55                  60

Pro Lys Phe Gln Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn
             65                  70                  75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
         80                  85                  90

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 768 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTCAGCAGCG TGACATCTGA GGACACCGCG GTCTAT 36

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 769 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ATAGACCGCG GTGTCCTCAG ATGTCACGCT GCTGAG 36

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..372
(D) OTHER INFORMATION: /note= "pBAG207 insert: SSE heavy chain variable region"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide

(B) LOCATION: 1..372

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..372

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG          48
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
 1               5                  10                  15

ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT AAA GAC ACC          96
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT         144
Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC CCG AAG TTC         192
Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe
    50                  55                  60

CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AGC AAC CAG TTC AGC         240
Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn Gln Phe Ser
65                  70                  75                  80

CTG AGA CTC AGC AGC GTG ACA TCT GAG GAC ACC GCG GTC TAT TAT TGT         288
Leu Arg Leu Ser Ser Val Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC TTC TGG GGC         336
Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly
            100                 105                 110

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC                         372
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp Pro Lys Phe
50 55 60

Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp Phe Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
115 120

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 704 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TGCACTGGGT GAAACAGCGA CCTGGACGAG G 31

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 705 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CCTCGTCCAG GTCGCTGTTT CACCCAGTGC A 31

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG185 insert: KRS heavy chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT      48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                 -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA      96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
            1               5                  10
```

```
CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT        144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAT ATG CAC TGG GTG AAA CAG CGA CCT GGA CGA GGT CTT        192
Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Gly Arg Gly Leu
 30                  35                  40                  45

GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC        240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                 50                  55                  60

CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AGC AAC        288
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
             65                  70                  75

CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC        336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
         80                  85                  90

TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC        384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC            429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19                 -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
                 1                   5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
     15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Gly Arg Gly Leu
 30                  35                  40                  45
```

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
50 55 60

Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
65 70 75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
80 85 90

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
95 100 105

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 745 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TGACCTGCAC CGCGTCTGGC TTCAAC 26

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 746 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TTGAAGCCAG ACGCGGTGCA GGTCAG 26

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG195 insert: AS heavy chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT        48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                 -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA        96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
            1               5                  10

CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GCG TCT GGC TTC AAC ATT       144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT       192
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30                  35                  40                  45

GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC       240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                50                  55                  60
```

```
CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AGC AAC      288
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
                65                  70                  75

CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC      336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
            80                  85                  90

TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC      384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
        95                  100                 105

TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC          429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19                 -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
                1                   5                  10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile
        15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
    30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                50                  55                  60

Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn
                65                  70                  75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
            80                  85                  90

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
        95                  100                 105
```

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 915 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TATTATTGTG CAAGAGGAAT GTGGGTATC 29

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 917 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ATACCCACAT TCCTCTTGCA CAATAATAG 29

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 918 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CTGCACCGTG TCTGGCTTCA CCTTCAGCGA CACCTATATG C 41

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 919 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GCATATAGGT GTCGCTGAAG GTGAAGCCAG ACACGGTGCA G 41

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 697 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGTGTCCACT CCAGCATCGT GATGACCCAG A 41

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 698 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TCTGGGTCAT CACGATGCTG GAGTGGACAC C 41

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 386 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..386

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..386

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG198 insert: VK2 (SVMDY) light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC GGT 48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19 -15 -10 -5

```
GTC CAC TCC AGC ATC GTG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC          96
Val His Ser Ser Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
                1               5                  10

AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT CAG AGT GTG         144
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val
     15                  20                  25

ACT AAT GAT GTA GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG         192
Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30                  35                  40                  45

CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT GGT GTG CCA GAT AGA         240
Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg
                50                  55                  60

TTC AGC GGT AGC GGT TAT GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC         288
Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
            65                  70                  75

CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC         336
Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser
        80                  85                  90

TCT CCG TAC ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGT AAG TG      386
Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys
     95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19                 -15                 -10                  -5

Val His Ser Ser Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
                1               5                  10

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val
     15                  20                  25

Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30                  35                  40                  45
```

Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg
50 55 60

Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
65 70 75

Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser
80 85 90

Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys
95 100 105

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 803 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGTGTCCACT CCGACATCCA GATGACCCAG AG 32

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of 804 oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CTCTGGGTCA TCTGGATGTC GGAGTGGACA CC 32

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 386 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..386

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..386

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG197 insert: VK3 (DQMDY) light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```
ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC GGT        48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19             -15                 -10                 -5

GTC CAC TCC GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC        96
Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
               1                 5                  10

AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT CAG AGT GTG       144
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val
     15                  20                  25

ACT AAT GAT GTA GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG       192
Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30                  35                  40                  45

CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT GGT GTG CCA GAT AGA       240
Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg
                 50                  55                  60
```

```
TTC AGC GGT AGC GGT TAT GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC      288
Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
            65                  70                  75

CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC      336
Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser
        80                  85                  90

TCT CCG TAC ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGT AAG TG   386
Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys
    95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19                 -15                 -10                  -5

Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
                1                   5                  10

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val
    15                  20                  25

Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
30                  35                  40                  45

Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg
                50                  55                  60

Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
            65                  70                  75

Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser
        80                  85                  90

Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys
    95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..429
(D) OTHER INFORMATION: /note= "pMDR1023 insert: PDLN heavy chain variable region"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..429

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```
ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT        48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19             -15                 -10                 -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG TCC GGT GCT GAA GTT GTT AAA        96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Ala Glu Val Val Lys
              1                 5                  10

CCG GGT TCC TCC GTT AAA CTG TCC TGC AAA GCT TCC GGT TTC AAC ATC       144
Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile
     15                  20                  25

AAA GAC ACC TAC ATG CAC TGG GTT AAA CAG CGT CCG GGT CAG GGT CTG       192
Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

GAA TGG ATC GGT CGT ATC GAC CCG GCT TCC GGT GAC ACC AAA TAC GAC       240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                 50                  55                  60

CCG AAA TTC CAG GTT AAA GCT ACC ATC ACC GCT GAC GAA TCC ACC TCC       288
Pro Lys Phe Gln Val Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Ser
             65                  70                  75

ACC GCT TAC CTG GAA CTG TCC TCC CTG CGT TCC GAA GAC ACC GCT GTT       336
Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
         80                  85                  90
```

```
TAC TAC TGC GCT GAC GGT ATG TGG GTT TCC ACC GGT TAC GCT CTG GAC        384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

TTC TGG GGT CAG GGT ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC        429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
-19                 -15                 -10                  -5

Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Ala Glu Val Val Lys
                 1                   5                  10

Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile
     15                  20                  25

Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
                    50                  55                  60

Pro Lys Phe Gln Val Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Ser
                65                  70                  75

Thr Ala Tyr Leu Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            80                  85                  90

Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp
     95                  100                 105

Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser
110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 383 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..376
(D) OTHER INFORMATION: /note= "pMDR1025 insert: PDLN light chain variable region"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58..376

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..376

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

```
ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC GGT        48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19             -15                 -10                 -5

GTT CAC TCC ATC GTT ATG ACC CAG TCC CCG GAC TCC CTG GCT GTT TCC        96
Val His Ser Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser
               1               5                  10

CTG GGT GAA CGT GTT ACC ATC AAC TGC AAA GCT TCC CAG TCC GTT ACC       144
Leu Gly Glu Arg Val Thr Ile Asn Cys Lys Ala Ser Gln Ser Val Thr
     15                  20                  25

AAC GAC GTT GCT TGG TAC CAG CAG AAA CCG GGT CAG TCC CCG AAA CTG       192
Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu
 30                  35                  40                  45

CTG ATC TAC TAC GCT TCC AAC CGT TAC ACC GGT GTT CCG GAC CGT TTC       240
Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe
                 50                  55                  60

TCC GGT TCC GGT TAC GGT ACC GAC TTC ACC TTC ACC ATC TCC TCC GTT       288
Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val
             65                  70                  75

CAG GCT GAA GAC GTT GCT GTT TAC TAC TGC CAG CAG GAC TAC TCC TCC       336
Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asp Tyr Ser Ser
         80                  85                  90
```

```
CCG TAC ACC TTC GGT GGT GGT ACC AAA CTG GAG ATC TAA GGA TCC TC        383
Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  *
 95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-19              -15                 -10                  -5

Val His Ser Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser
               1               5                  10

Leu Gly Glu Arg Val Thr Ile Asn Cys Lys Ala Ser Gln Ser Val Thr
     15                  20                  25

Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu
 30                  35                  40                  45

Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe
                 50                  55                  60

Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val
             65                  70                  75

Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asp Tyr Ser Ser
         80                  85                  90

Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  *
 95                  100                 105
```

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "Oligo 370-119 corresponding to 58-117 VH-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CAGGTTCAGC TGCAGGAGTC CGGTGCTGAA GTTGTTAAAC CGGGTTCCTC CGTTAAACTG 60

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-120 corresponds to 118-177 VH-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

TCCTGCAAAG CTTCCGGTTT CAACATCAAA GACACCTACA TGCACTGGGT TAAACAGCGT 60

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-121 corresponds to 178-237 VH-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CCGGGTCAGG GTCTGGAATG GATCGGTCGT ATCGACCCGG CTTCCGGTGA CACCAAATAC 60

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-122 corresponds to 238-303 VH-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GACCCGAAAT TCCAGGTTAA AGCTACCATC ACCGCTGACG AATCCACCTC CACCGCTTAC 60

CTGGAA 66

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-123 corresponds to 304-366 VH-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CTGTCCTCCC TGCGTTCCGA AGACACCGCT GTTTACTACT GCGCTGACGG TATGTGGGTT 60

TCC 63

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1

(D) OTHER INFORMATION: /note= "Oligo 370-124 corresponds to 367-420 VH-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

ACCGGTTACG CTCTGGACTT CTGGGGTCAG GGTACCACGG TCACCGTTTC CTCC 54

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-125 corresponds to reverse VH-PDLN 420-358"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GGAGGAAACG GTGACCGTGG TACCCTGACC CCAGAAGTCC AGAGCGTAAC CGGTGGAAAC 60

CCA 63

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-126 corresponds to reverse VH-PDLN 357-311"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

CATACCGTCA GCGCAGTAGT AAACAGCGGT GTCTTCGGAA CGCAGGG 47

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-127 corresponds to reverse VH-PDLN 310-244"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

AGGACAGTTC CAGGTAAGCG GTGGAGGTGG ATTCGTCAGC GGTGATGGTA GCTTTAACCT 60

GGAATTT 67

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-128 corresponds to reverse VH-PDLN 243-186"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CGGGTCGTAT TTGGTGTCAC CGGAAGCCGG GTCGATACGA CCGATCCATT CCAGACCCTG 60

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-129 corresponds to reverse VH-PDLN 185-124"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

ACCCGGACGC TGTTTAACCC AGTGCATGTA GGTGTCTTTG ATGTTGAAAC CGGAAGCTTT 60

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-130 corresponds to reverse VH-PDLN 123-58"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GCAGGACAGT TTAACGGAGG AACCCGGTTT AACAACTTCA GCACCGGACT CCTGCAGCTG 60

AACCTG 66

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-131 corresponds to 1-58 VK-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

AGCTTACCAT GGGTTGGTCC TGCATCATCC TGTTCCTGGT TGCTACCGCT ACCGGTGTTC 60

ACTCCA 66

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-132 corresponds to 59-124 VK-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TCGTTATGAC CCAGTCCCCG GACTCCCTGG CTGTTTCCCT GGGTGAACGT GTTACCATCA 60

ACTGCA 66

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-133 corresponds to 125-190 VK-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

AAGCTTCCCA GTCCGTTACC AACGACGTTG CTTGGTACCA GCAGAAACCG GGTCAGTCCC 60

CGAAAC 66

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-134 corresponds to 191-256 VK-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TGCTGATCTA CTACGCTTCC AACCGTTACA CCGGTGTTCC GGACCGTTTC TCCGGTTCCG 60

GTTACG 66

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-135 corresponds to 257-322 VK-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GTACCGACTT CACCTTCACC ATCTCCTCCG TTCAGGCTGA AGACGTTGCT GTTTACTACT 60

GCCAGC 66

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "oligo 370-136 corresponds to 323-376 VK-PDLN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

AGGACTACTC CTCCCCGTAC ACCTTCGGTG GTGGTACCAA ACTGGAGATC TAAG 54

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "oligo 370-137 corresponds to reverse VK-PDLN 380-318"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GATCCTTAGA TCTCCAGTTT GGTACCACCA CCGAAGGTGT ACGGGGAGGA GTAGTCCTGC 60

TGG 63

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-138 corresponds to reverse VK-PDLN 317-252"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CAGTAGTAAA CAGCAACGTC TTCAGCCTGA ACGGAGGAGA TGGTGAAGGT GAAGTCGGTA 60

CCGTAA 66

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-139 corresponds to reverse VK-PDLN 251-186"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

CCGGAACCGG AGAAACGGTC CGGAACACCG GTGTAACGGT TGGAAGCGTA GTAGATCAGC 60

AGTTTC 66

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-140 corresponds to reverse VK-PDLN 185-120"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GGGGACTGAC CCGGTTTCTG CTGGTACCAA GCAACGTCGT TGGTAACGGA CTGGGAAGCT 60

TTGCAG 66

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-141 corresponds to reverse VK-PDLN 119-54"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TTGATGGTAA CACGTTCACC CAGGGAAACA GCCAGGGAGT CCGGGGACTG GGTCATAACG 60

ATGGAG 66

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Oligo 370-142 corresponds to reverse VK-PDLN 53-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

TGAACACCGG TAGCGGTAGC AACCAGGAAC AGGATGATGC AGGACCAACC CATGGTA 57

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of VK1-DQL primer 307-247"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

ACCGCTACCG GTGTTCACTC CGACATCCAG CTGACCCAGA GCCCAAGCAG C 51

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequecne of VK1-DQL primer 370-210"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

CTGAGGATCC AGAAAGTGCA CTTACGTTTG ATTTCCACCT TGGTCCCTTG GCCGAA 56

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of VK2-SVMDY primer 370-269"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CTCTCCACCG GTGTCCACTC CAGCATCGTG ATGACCCAGA GCCCAAGCAG C 51

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "DNA sequence of VK3-DQMDY primer 370-268"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CTCTCCACCG GTGTCCACTC CGACATCCAG ATGACCCAGA GCCCAAGCAG C 51

WHAT IS CLAIMED IS:

1. A recombinant antibody molecule comprising antigen binding regions derived from the heavy or light chain variable regions of an anti-VLA4 antibody.

2. A humanized recombinant antibody molecule having specificity for VLA4 and having an antigen binding site wherein at least one of the complementarity determining regions (CDR) of the variable regions are derived from a non-human anti-VLA4 antibody.

3. A humanized recombinant heavy chain according to claim 2 comprising non-human CDRs at positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) (Kabat numbering).

4. A humanized recombinant heavy chain according to claim 3 comprising non-human residues at framework positions 27-30 (Kabat numbering).

5. A humanized recombinant heavy chain according to claim 4 comprising additional non-human residues at framework position 75 (Kabat numbering).

6. A humanized recombinant heavy chain according to claim 5 comprising additional non-human residues at framework position(s) 77-79 or 66-67 and 69-71 or 84-85 or 38 and 40 or 24.

7. A humanized recombinant light chain according to claim 2 comprising non-human CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3).

8. A humanized recombinant light chain according to claim 7 comprising non-human residues at framework positions 60 and 67.

9. A humanized recombinant antibody molecule comprising at least one antibody heavy chain according to claim 3 and at least one antibody light chain according to claim 7.

10. A humanized recombinant antibody molecule according to claim 7 wherein the non-human CDRs are derived from the HP1/2 murine monoclonal antibody.

11. DNA encoding an antibody heavy chain according to claim 3.

12. DNA encoding an antibody light chain according to claim 7.

13. DNA encoding an antibody molecule according to claim 10.

14. A vector comprising DNA according to claim 11.

15. A vector comprising DNA according to claim 12.

16. A vector comprising DNA according to claim 13.

17. An expression vector comprising DNA encoding an antibody heavy chain according to claim 3 in operative combination with DNA encoding an antibody light chain according to claim 7.

18. An expression vector comprising DNA encoding an antibody molecule according to claim 10.

19. Host cells transformed with a vector according to claim 14 and a vector according to claim 15.

20. Host cells transformed with a vector according to claim 16.

21. A process for the production of a humanized recombinant anti-VLA4 antibody comprising:

(a) producing an expression vector comprising an operon having a DNA sequence encoding an antibody heavy or light chain wherein at least one of the CDRs of the variable domain are derived from a non-human anti-VLA4 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(b) producing an expression vector comprising an operon having a DNA sequence encoding a complementary antibody light or heavy chain wherein at least one of the CDRs of the variable domain are derived from a non-human anti-VLA4 antibody and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(c) transfecting a host cell with each vector; and

(d) culturing the transfected cell line to produce the humanized recombinant anti-VLA4 antibody molecule.

22. A process according to claim 21 wherein the DNA sequence encoding the heavy chain and the light chain comprise the same vector.

23. A therapeutic composition comprising an antibody molecule, or a fragment thereof, according to claim 1 in combination with a pharmaceutically acceptable diluent, excipient or carrier.

24. A diagnostic composition comprising an antibody molecule, or a fragment thereof, according to claim 1 in a detectably labelled form.

25. A method of treatment comprising administering an effective therapeutic amount of an antibody according to claim 1 to a human or animal subject.

26. A method for treating inflammation resulting from a response of a specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the inflammation, wherein the anti-inflammatory agent is an antibody according to claim 1.

27. A humanized recombinant anti-VLA4 antibody molecule having the characteristics of an antibody which comprises a humanized heavy chain comprising a variable heavy chain region selected from the group consisting of V_H - STAW (SEQ ID NO:39), V_H - KAITAS (SEQ ID NO:43), V_H - SSE (SEQ ID NO:47), V_H - KRS (SEQ ID NO:51), and V_H - AS (SEQ ID NO: 55), in combination with a humanized light chain comprising a light chain variable region selected from the group consisting of VK - DQL (SEQ ID NO: 31), VK2 - SVMDY (SEQ ID NO: 63), and VK3 - DQMDY (SEQ ID NO: 67).

28. DNA encoding the humanized heavy chain and the humanized light chain according to claim 27.

29. A vector comprising DNA according to claim 28.

30. An expression vector comprising DNA encoding an antibody molecule according to claim 27.

31. Host cells transformed with a vector according to claim 29.

32. Host cells transformed with a vector according to claim 30.

33. Host cells according to claim 32 that are ATCC CRL 11175.

34. A humanized recombinant anti-VLA4 antibody molecule having a potency from about 20% to about 100% of the potency of an antibody which comprises a humanized heavy chain comprising a variable heavy chain region of V_H - AS (SEQ ID NO: 55), in combination with a humanized light chain comprising a light chain variable region of VK2 - SVMDY (SEQ ID NO: 63).

35. A therapeutic composition comprising an antibody molecule, or a fragment thereof, according to claim 27 or 34 in combination with a pharmaceutically acceptable diluent, excipient or carrier.

36. A diagnostic composition comprising an antibody molecule, or a fragment thereof, according to claim 27 or 34 in a detectably labelled form.

37. A method of treatment comprising administering an effective amount of an antibody according to claim 27 or 34 to a human or animal subject.

38. A method for treating inflammation resulting from a response of a specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress the inflammation, wherein the anti-inflammatory agent is an antibody according to claim 27 or 34.

39. A humanized recombinant anti-VLA4 antibody molecule that is the antibody produced by ATCC CRL 11175 or an antibody having the characteristics of the antibody produced by ATCC CRL 11175.

40. A humanized recombinant anti-VLA4 antibody molecule that has a potency from about 20% to about 100% of the potency of the antibody produced by ATCC CRL 11175.

41. A humanized recombinant anti-VLA4 antibody molecule that has a potency from about 20% to about 100% of the potency of the antibody produced by the murine monoclonal antibody HP1/2.

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(54) Title: RECOMBINANT ANTI-VLA4 ANTIBODY MOLECULES

(57) Abstract

The present invention discloses recombinant anti-VLA4 antibody molecules, including humanized recombinant anti-VLA4 antibody molecules. These antibodies are useful in the treatment of specific and non-specific inflammation, including asthma and inflammatory bowel disease. In addition, the humanized recombinant anti-VLA4 antibodies disclosed can be useful in methods of diagnosing and localizing sites of inflammation.

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INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 94/00266

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO,A,94 11027 (BOARD OF REGENTS OF UNIVERSITY OF WASHINGTON) 26 May 1994 see Example 6 and SEQ ID NO:6, NO:8, NO: 10 ---	1-25, 27-37, 39-41
P,X	WO,A,93 13798 (BIOGEN) 22 July 1993 see page 9 and claims ---	1
Y	EP,A,0 330 506 (DANA-FARBER CANCER INSTITUTE) 30 August 1989 see Page 2, Lines 43-51, Page 4, Lines 41-54 and claims 8-10 ---	1-10
	-/--	

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[X] Patent family members are listed in annex.

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Date of the actual completion of the international search
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